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Information gathering and conflict resolution in *Polistes* wasps

Jonathan Philip Green

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Declaration

The design and data collection for the study presented in Chapter 4 were undertaken in collaboration with Dr. Elli Leadbeater at the Institute of Zoology and Professor Jeremy Field at the University of Sussex. However, the particular analyses undertaken in that chapter, as well as the interpretations drawn from the data, are my own.

I certify that, with the above qualification, the work carried out in this thesis is entirely my own, and that any help provided by other individuals with data collection and analysis is fully acknowledged.

In addition, I certify that this thesis has not been, and will not be, submitted in whole or in part to another university for the award of any other degree.

Signature:

Jonathan Philip Green

UNIVERISTY OF SUSSEX

JONATHAN PHILIP GREEN, DOCTOR OF PHILOSOPHY

INFORMATION GATHERING AND CONFLICT RESOLUTION IN *POLISTES* WASPS

SUMMARY

Signals are used to communicate resource-holding potential (RHP) to rivals during contests across a wide range of taxa. A controversial subset of RHP signals are status signals. In the last decade, research on North American populations of the paper wasp *Polistes dominulus* has provided evidence for a visual status signal based on variable clypeal patterns. However, observations of *P. dominulus* in its native European range indicate that the use of status signals across populations might be limited in this species. In Part I of this thesis (Chapters 3-5), I investigate status signalling in a Spanish population of *P. dominulus*. Using choice experiments, I show that clypeal patterns do not signal RHP in the Spanish population. Using large-scale field observations and microsatellite sequencing, I then show that patterns do not reflect individual quality in the wild. Together, these results strongly suggest that the clypeal pattern does not function in conflict resolution in the Spanish population. I conclude Part I by exploring the development of the clypeal patterns. I show that pattern expression is strongly temperature-dependent. This finding may provide an explanation for the variation in the signal value of clypeal patterns between populations.

Contests among paper wasps are not limited to conspecific interactions, but may involve interactions with social parasites. In Parts II and III of this thesis (Chapters 6-7), I explore interactions between *P. dominulus* and the social parasite *P. semenowi* in the contexts of nest usurpation and conflict over reproduction. By experimentally staging usurpation contests, I show that neither parasites nor hosts gather information about rivals during nest usurpation. I then compare reproduction in parasitised and unparasitised colonies to test the predictions of competing models of reproductive skew. Incomplete control models receive qualified support; however, assumptions of skew models about players' information gathering abilities are questioned.

Publications arising from this thesis

First-authored publications:

Green, J. P. & Field, J. 2011. Assessment between species: information gathering in usurpation contests between a paper wasp and its social parasite. *Animal Behaviour*, **81**, 1263-1269.

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In the course of my doctoral research, I have contributed to studies led by colleagues in the Social Evolution Research Group, which has resulted in shared authorship of the following papers:

Leadbeater, E., Carruthers, J. M., **Green, J. P**., van Heusden, J. & Field, J. Unrelated helpers in a primitively eusocial wasp: is helping tailored towards direct fitness? *Plos One*, **5**, e11997.

Leadbeater, E., Carruthers, J. M., **Green, J. P**., Rosser, N. & Field, J. 2011. Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science*, **333**, 874-876.

Copies of all four publications are included at the end of this thesis as supporting material (see Appendix D).

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Chapter 1: General Introduction

1.1 Information gathering and conflict resolution

Animals are frequently required to make decisions in response to important stimuli (Mappes & Stevens 2010). In order to make optimal decisions, i.e. those that will maximise their fitness, animals must gather information about their internal state and/or relevant aspects of the external environment (Danchin *et al*. 2008). Such information acts to reduce the uncertainty about the fitness consequences of alternative behavioural responses, and in doing so allows an individual to select the best strategy of the options available (Dall *et al*. 2005). Information gathering is therefore a topic of fundamental importance in behavioural ecology, which is reflected in the enormous volume of research devoted to understanding how animals acquire relevant information, how this information is processed and how it informs behavioural responses (Bradbury & Vehrencamp 1998; Dall *et al*. 2005; Danchin *et al*. 2008; Mappes & Stevens 2010).

A vital component of an animal's external environment is other animals, and it is often essential that individuals of the same or different species gather information about one another. For example, it is advantageous to a predator to gather information about the whereabouts or suitability of a particular prey item. Similarly, selection should favour adaptations in the prey that allow it to detect and respond to the threat of predation (Mappes & Stevens 2010). Information gathering is also important in numerous other contexts, including mate choice, selection of breeding and foraging sites, and in the resolution of conflicts between individuals (e.g. Maynard Smith & Harper 2003; Valone 2007; Arnott & Elwood 2008, 2009).

Conflicts between animals are ubiquitous in nature, and arise as a consequence of disagreement over the allocation of resources between non-genetically identical individuals. Among animals, the outcome of conflict is important in determining access to resources, including those necessary for reproduction (Andersson 1994; Clutton-Brock 2007). To identify the optimal behavioural response in situations of conflict, an individual could gather information about one or more important features of the

conflict, including the value of the contested resource; its own ability to acquire the resource, and the ability of its opponent to acquire the resource (Enquist & Leimar 1987; Arnott & Elwood 2008, 2009). This information then allows an individual to assess the relative costs and benefits of competing versus withdrawing and thereby relinquishing the resource (Arnott & Elwood 2009). Information gathering thus allows individuals to avoid the potential costs of escalated fighting, which in some cases can be severe (Clutton-Brock *et al*. 1979; Mann *et al*. 2001; Arnott & Elwood 2009). In general, the importance of information in the resolution of conflict is thought to exert a strong selective pressure for effective information gathering during conflicts over resources (Bradbury & Vehrencamp 1998; Maynard Smith & Harper 2003).

In this thesis, I explore three examples of information gathering and conflict resolution in *Polistes* wasps. In Part I, I explore signalling during contests between conspecifics in the wasp *Polistes dominulus*. In Part II, I investigate the potential for information gathering in heterospecific contests between *P. dominulus* and the social parasite *P. semenowi*. Finally, in Part III, I explore mechanisms of reproductive partitioning within *P. dominulus* groups, and consider the importance of information gathering in the distribution of reproduction among group members. In the following sections, I introduce each of these topics in turn, before concluding this chapter with a summary of research aims.

1.2 Information gathering during animal contests

Contests over resources can inflict significant costs, in terms of both the time and energy expended in competition, as well as the risk of injury or death associated with escalated fighting (e.g. Clutton-Brock *et al*. 1979; Mann *et al*. 2001; Briffa & Sneddon 2007). In the absence of any information about the likely outcome of the contest, fighting will escalate until one individual (the loser) withdraws or else is incapacitated. Consequently, both contestants will pay costs, whose magnitude depends on both the loser's motivation and the severity of the costs inflicted during fighting (Arnott & Elwood 2008, 2009). However, an extensive body of theory indicates that individuals can avoid paying these costs by gathering information about a rival's abilities at the

start of the contest, which can then be used to predict the likely winner without resorting to costly escalation (Maynard Smith & Parker 1976; Enquist 1985; Maynard Smith & Harper 1988; reviewed in Maynard Smith & Harper 2003; Searcy & Nowicki 2005).

The sources of information available to individuals about a rival's abilities can be separated in the first instance into signals and cues. Maynard Smith & Harper (2003) define a signal as 'any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved' (Maynard Smith & Harper 2003). In contrast, cues are features that provide information but that have not evolved for this purpose (Bradbury & Vehrencamp 1998). A common example of a cue in animal contests is body size, which can be used to infer the competitive abilities of a rival, but which has not evolved expressly for that purpose (Maynard Smith & Harper 2003). In Part I of this thesis, I focus on information gathering via the use of signals, which I describe in more detail below. However, as a recent review by Arnott & Elwood (2009) makes clear, cues are important in rival assessment, and the ability of animals to use cues such as body size to predict the outcome of contests has been the focus of recent tests of various game theoretical models of assessment (Arnott & Elwood 2009; see also Chapter 6).

1.2.1 Contest signals

Evolution of contest signals

During contests, individuals may communicate agonistic ability through the use of signals (see above). The evolution of agonistic signalling between conspecifics is generally considered to proceed via intrasexual selection (Clutton-Brock 2004). Under intrasexual selection, traits are favoured that confer an advantage in competition among members of one sex (usually males) over reproductive benefits (e.g. access to mating opportunities) (Andersson 1994). Evidence of intrasexual signalling is observed in a large number of species, in which signals are used in contests between males over access to females or other reproductive benefits e.g. territories (Clutton-Brock 2004).

However, a number of researchers have questioned whether intrasexual selection is sufficient to explain the occurrence of agonistic signalling in some species (e.g. West-Eberhard 1979; Tanaka 1996). Two observations in particular are argued to be inconsistent with the view of the evolution of such signals via intrasexual selection. First, in several avian and lizard species, agnostic signalling occurs not only in contests over reproduction but also in competition over food (Whitfield 1987; Whiting *et al*. 2003). Second, in a number of these species, the use of agonistic signals is not restricted to males but also occurs in competition between females (Senar 1999; Kraaijeveld *et al*. 2007). These observations have led researchers to argue that agonistic signals in these species have not evolved via intrasexual selection, but instead via a process of natural selection termed 'social selection' (West-Eberhard 1979; Tanaka 1996). According to these authors, agonistic signals evolving through social selection are favoured because of the benefits to both signallers and receivers in social contexts other than competition over reproduction (West-Eberhard 1979; Rohwer 1982; Tanaka 1996).

However, social selection have been criticised on a number of grounds (Kraaijeveld *et al*. 2007; Clutton-Brock 2009). First, it is often hard to distinguish between resources that confer reproductive benefits and those that do not (Kraaijeveld *et al*. 2007). For example, while contests over food do not offer immediate reproductive benefits, the outcome of such contests may nonetheless have important consequences for an individual's reproductive success through an effect on condition or survival. The difficulty in distinguishing sexual and non-sexual resources thus makes it challenging to identify examples of traits under social selection which could not alternatively be interpreted as being under sexual selection (Kraaijeveld *et al*. 2007). The occurrence of agonistic signalling among females has also been used as evidence for social selection rather than sexual selection. However, as Clutton-Brock (2009) notes, intrasexual selection is possible among females as well as males. In particular, there can be intense competition among females for resources necessary for reproduction (potentially including food, nest sites or social rank), which is expected to generate strong intrasexual selection among females (Clutton-Brock 2009). The occurrence of agonistic signalling among females is thus (mostly) consistent with sexual selection theory and does not appear to require the existence of a separate selection process (Clutton-Brock 2009; see also Kraaijeveld *et al*. 2007).

Thus, while the emphasis placed on social competition in the evolution of signalling is clearly important (e.g. West-Eberhard 1979), there currently appears to be no way of distinguishing traits evolving via social selection from those evolving via sexual selection (Kraaijeveld *et al*. 2007). For simplicity, I therefore follow Clutton-Brock (2009) in considering agonistic signalling to be under intrasexual selection in this thesis. Importantly, as Senar (2006) notes, nothing appears to be lost in this approach: regardless of whether sexual or social selection drives a given signalling system, the adaptive significance of signals remains the same: individuals benefit by assessing the agonistic ability of rivals, thereby avoiding costly escalation.

Signal function and reliability

Contest signals may provide information about several aspects of an individual's agonistic ability, including its motivation, aggressiveness and fight power, commonly referred to as resource-holding potential, or RHP (Parker 1974). Providing that this information is reliable (i.e. it accurately reflects the signaller's agonistic ability), the receiver will benefit from attending to the signal, as it can use this information to assess the benefits of continued fighting versus withdrawal from the contest (Arnott & Elwood 2009). Importantly, the signaller also benefits by providing this information, as it reduces the need for escalation, which would otherwise be necessary to reveal differences in agonistic ability between contestants (Maynard Smith & Harper 2003).

Yet it is not obvious why information sent by a signaller should be reliable. While the receiver benefits from accurate information about a signaller's state, the signaller is expected to signal at a level which maximises the benefits that it derives from the receiver's response (Dawkins & Krebs 1978). Where the evolutionary interests of receivers and signallers collide (e.g. in a contest over a fitness-enhancing resource), signals are vulnerable to exploitation by 'cheats' that provide misleading information in order to secure a particular response from receivers (Searcy & Nowicki 2005). The prospect for cheating has been studied extensively (e.g. Enquist 1985; Maynard Smith & Harper 1988; Owens & Hartley 1991; Johnstone & Norris 1993; Számadó 2011). During a contest, a cheat that signals a higher agonistic ability than is actually the case will receive the same benefits as an individual honestly signalling a high ability (in

terms of the effect on the behaviour of the receiver, which may respond in both cases by withdrawing from the contest), yet does not pay the cost associated with a genuinely high agonistic ability. In the absence of some mechanism to ensure signal honesty, ESS models of signalling predict that cheats will then spread at the expense of honest signallers, with the result that the signal no longer provides a reliable guide to agonistic ability (Searcy & Nowicki 2005).

Theoretical models of contest signalling predict that signals may be stable (i.e. honest) if there is some cost associated with sending signals that discourage low-quality individuals from signalling a high level of quality (Enquist 1985; Johnstone & Norris 1993; see also Grafen 1990; Számadó 2011). Alternatively, honesty may be maintained where low quality individuals are physically constrained from signalling at a high intensity (Maynard Smith & Harper 2003). Based on these contrasting mechanisms, two broad categories of contest signal can be distinguished: strategic signals, which impose costs that are disproportionately greater for low-quality individuals, thereby making cheating unprofitable (but not impossible), and indices, which are argued to be honest because they are unfakeable (Maynard Smith & Harper 2003; Searcy & Nowicki 2005).

Strategic signals can be further divided into handicaps (Zahavi 1975; Grafen 1990) and conventional (or 'status') signals, based on the type of cost that maintains honesty in the signal (Guilford & Dawkins 1995; Maynard Smith & Harper 2003). In the case of handicaps, honesty is maintained by costs associated with the production and expression of the signal, over and above the efficacy costs necessary to transmit the signal (Maynard Smith & Harper 2003). In the case of status signals, however, costs are incurred through interactions with receivers (Guilford & Dawkins 1995). Importantly, for both handicaps and status signals to be honest, high-quality individuals must be better able to support these costs than individuals of lower quality (Searcy & Nowicki 2005). Beyond this common requirement, however, the two types of signal differ in important ways. In the case of handicaps, it is the investment in the signal that carries the cost, meaning that the intensity of the signal provides specific information about an individual's ability to bear this cost i.e. there is a causal link between quality and signal intensity (Zahavi 1975; Searcy & Nowicki 2005). In contrast, the costs maintaining honesty in status signals arise through interactions with receivers and not through

investment in the signal (Searcy & Nowicki 2005). Thus, in the case of status signals, there is no requirement that the intensity of the signal be causally related to the quality that is signalled (Guilford & Dawkins 1995). Nevertheless, signal intensity is a reliable indicator of quality as only high-quality individuals are able to support the receiver-dependent costs (or 'social costs') associated with a high signal intensity (Guilford & Dawkins 1995; Maynard Smith & Harper 2003). In this thesis, I focus on the use of status signals in rival assessment. Below, I briefly review the evidence for status signals, including the evidence for social costs maintaining signal honesty.

1.2.2 Status signalling

Introduction

Research into the use of status signals in animal contests encompasses a large number of empirical studies on a variety of taxa, as well as a series of theoretical models that explore the conditions under which such signals can evolve to be honest. In-depth reviews of this research can be found in Senar (1999, 2006), Maynard Smith & Harper (2003), Whiting *et al*. (2003), Searcy & Nowicki (2005) and Számadó (2011). The aim of this section is to provide a brief outline of empirical and theoretical research into status signalling, focusing in particular on the difficulties associated with experimental demonstrations of both status signals and the social costs argued to prevent cheating.

The existence of status signals was first mooted by Rohwer (1975), who found that the size of the black chest bib was positively correlated with hierarchical rank within flocks of Harris' sparrows (*Zonotrichia querula*), and that differences in bib size between individuals predicted the outcome of contests (Rohwer 1975). Subsequent studies on other passerines also reported a positive correlation between plumage coloration and social status, leading researchers to suggest that plumage signals agonistic ability in these species (Senar 1999, and references therein). However, as noted by Roper (1986), a positive association between plumage and status does not necessarily imply that plumage signals agonistic ability; rather, individuals may use other cues that are correlated with plumage to assess status (Roper 1986). Therefore, to test whether

plumage functions as a signal of status, a number of studies tested responses to experimentally altered plumage.

In the first of these experiments, Rohwer (1977) found that sparrows that had been dyed or bleached to alter the size of the bib received increased aggression when returned to their social groups. This result demonstrates that changes to an individual's plumage can effect changes in another individual's behaviour, consistent with plumage having a signal function. However, a similar result would also be expected if birds were able to recognise flock-mates individually and react aggressively to the re-introduction of individuals made to appear unfamiliar by the manipulations (Shields 1977). Conversely, the potential for individual recognition also means that the failure of manipulations to elicit responses from group members cannot be considered as proof that the manipulated trait does not signal status if receivers recognise the true status of the manipulated individual based on other cues (Senar 1999).

Subsequent demonstrations of status signalling have therefore attempted to avoid the problems associated with signaller-receiver familiarity by testing receiver responses to unfamiliar signallers (reviewed in Senar 1999). For example, Järvi & Bakken (1984) tested the signal function of the black breast stripe in great tits (*Parus major*) using a model bird placed on a feeder. Receiver responses towards the model were found to depend on the difference in the width of the stripe between the receiver and the model: birds with wider stripes than the model approached the model aggressively, while those with smaller stripes were more submissive, indicating that stripe width communicates agonistic ability (Järvi & Bakken 1984). In a second study, Senar & Camerino (1998) allowed Eurasian siskins (*Carduelis spinus*) to choose between two food sources, one 'guarded' by a rival with an experimentally-increased chest bib, and the other by a rival with an experimentally-reduced bib. The authors found a preference for feeding near the guard with the smaller bib, indicating that the bib provides information about a rival that is used to make decisions in a competitive context (Senar & Camerino 1998).

Using these and other experimental designs, evidence of status signalling based on patches of colour has been obtained for a number of avian species (Senar 1999, 2006,

and references therein), as well as in several lizards (Whiting *et al*. 2003) and insects (Beani & Turillazzi 1996; Tibbetts & Lindsay 2008). However, the use of status signals is by no means universal: research on a number of other species of birds and lizards exhibiting polymorphism in body coloration have found no evidence that such coloration communicates agonistic ability between conspecifics (reviewed in Whiting *et al*. 2003). Nonetheless, the evidence to date suggests that, in some species at least, patches of colour with no logical connection to fighting ability can nevertheless evolve to signal an individual's aggression or RHP during contests[1] (Whitfield 1987; Maynard Smith & Harper 2003).

Status signals based on patches of colour are puzzling for two reasons. First, as noted above, such signals do not appear to bear any relation to agonistic ability, meaning that an individual is not physically constrained to signal at a particular intensity, unlike the case of indices (Maynard Smith & Harper 2003; but see Ducrest *et al*. 2008). Second, in contrast with strategic signals, status signals are assumed to have few production costs, in terms of investment in pigments underlying the signal (Maynard Smith & Harper 2003). This is based largely on the observation that status in many avian species is signalled either via melanin-based plumage (e.g. the black bibs of the Harris' sparrow and siskin), which has traditionally been considered cheaper to produce than other kinds of pigments (e.g. carotenoids) (Jawor & Breitwisch 2003; but see Griffith *et al*. 2006), or else via white plumage lacking pigment altogether (e.g. the white forehead patch of the collared flycatcher (*Ficedula albicollis*) (Qvarnström 1997)). Together, the arbitrary nature of status signals with respect to agonistic ability and the perceived lack of cost in signal production have presented a special difficulty to researchers for understanding how such signals can reliably signal aggressiveness or RHP. In the absence of physical constraints or costs of signal production, researchers have instead argued that social costs arising from interactions with receivers can maintain reliability in status signalling systems (see above). Below, I describe these social costs, and provide a brief review of evidence for social costs stabilising status signals in animal contests.

[1] Although the focus of this thesis is on visual status signals (i.e. those based on body coloration), it appears that animals can signal status along other sensory channels. For example, there is evidence for olfactory status signalling in rock lizards (Martin *et al*. 2007), while in banded wrens Molles & Vehrencamp (2001) have argued that song functions as an auditory status signal in territorial contests.

Social costs: theory and evidence

The stability of signals lacking production costs has been explored in several game theoretical models. In an early model of contest signalling, Enquist (1985) showed that signals lacking production costs could nonetheless evolve to be honest indicators of aggression, so long as the cost incurred by a weak individual in a fight with a strong individual exceeds the potential benefit derived from fighting. A similar result was obtained by Maynard Smith & Harper (1988), who showed that honesty was possible provided that the cost of fighting outweighed the value of the resource and that a dishonest signaller paid a cost equivalent to the level of aggressiveness that it signals. Together, these results indicate that low-cost signals can be used in contests over low-value resources, and that such signals can be stable where dishonest signallers (i.e. low-status individuals signalling a high status) risked incurring costs from escalated fights against genuine high-status individuals (Enquist 1985; Maynard Smith & Harper 1988).

Fundamental to the stability of cost-free signals in both Enquist's (1985) and Maynard Smith & Harper's (1988) models is the assumption that dishonest signallers receive costs equivalent to their level of signalling (Maynard Smith & Harper 1988; Számadó 2011). However, efforts by researchers to identify the mechanism that generates this cost have met with considerable difficulty (Senar 1999). The first attempt to identify this cost was the 'social control' hypothesis of Rohwer (1977), which posited that escalation between individuals signalling at a similar intensity ('like versus like aggression') could prevent individuals from signalling a dishonestly high status (Rohwer 1977). However, this hypothesis has not received widespread support, and in particular seems unlikely to account for the stability of status signals in a number of species where like versus like aggression does not seem to occur (Senar 1999).

A second hypothesis for the existence of social costs that prevent dishonest signalling is the 'incongruence hypothesis', also proposed by Rohwer (1977). Under this hypothesis, receivers direct aggression towards dishonest signallers when they detect an incongruity between the intensity of the signal and other behavioural cues revealed during aggressive interactions (Rohwer 1977; Rohwer & Rohwer 1978). Thus, a weak individual signalling a high status that attempted to flee from an escalating conflict

would receive aggression from rivals based on the mismatch between the signal and the behaviour (Rohwer 1977). Initial support for this hypothesis came from experiments on birds by Rohwer & Rohwer (1978) and Jarvi *et al*. (1987) in which plumage and aggression levels were simultaneously altered using paint and injections of testosterone. Individuals receiving this treatment rose in status following reintroduction into the flock, whereas individuals in which either plumage or aggression were altered did not rise in status. However, the methods used in these experiments have been criticised (Senar 1999), and, as with the social control hypothesis, the incongruence hypothesis has enjoyed only limited support (Senar 1999; but see Tibbetts & Izzo 2010).

The lack of strong empirical support aside, neither the incongruence hypothesis nor the social control hypothesis appear able to explain how an individual is forced to pay costs equivalent to its level of signalling (Whitfield 1987; Senar 1999). Escalation between individuals signalling at similar levels is argued to prevent cheating under the social control hypothesis. However, it is not clear what prevents a dishonest signaller from withdrawing when the threat of escalation materialises, rather than continuing to fight and incurring costs (Whitfield 1987). Under the incongruence hypothesis, a dishonest signaller is specifically targeted by a receiver and therefore presumably does not have an option to withdraw and escape the receiver's aggression (Senar 1999). However, this begs the question of why receivers deliberately target dishonest signallers, when such behaviour is likely to impose costs on the receiver (Maynard Smith & Harper 1988). In the absence of a benefit to the receiver, such behaviour would appear to constitute spite, and would consequently not be favoured by selection (Clutton-Brock & Parker 1995). Recently, Tibbetts & Izzo (2010) have suggested that this aggression may not represent a specific act of punishment, but may instead be a consequence of a receiver's decision to escalate the fight in a bid to determine the true status of a rival whose signal and behaviour are in conflict (Tibbetts & Izzo 2010). However, it remains unclear whether social costs generated in this way would be sufficient to deter dishonest signallers, particularly if the signaller was able to withdraw in the early stages of the fight.

Contest-independent costs

An important focus of debate surrounding the stability of status signals is whether social costs alone are sufficient to prevent cheating (Johnstone & Norris 1993; Maynard Smith & Harper 2003; Számadó 2011). While the original models of Enquist (1985) and Maynard Smith & Harper (1988) considered costs arising only during contests, more recent models of aggressive signalling by Owens & Hartley (1991) and Johnstone & Norris (1993) suggest that signal stability also requires a contest-independent cost to aggression (i.e. a cost that aggressive individuals pay a higher cost outside of any particular fights). In the absence of this cost, Owens & Hartley (1991) and Johnstone & Norris (1993) argue that the system is vulnerable to cheating by an individual that dishonestly signals a lower level of aggression (termed a 'modest' or 'Trojan' strategy). Such a strategy would always win against individuals honestly signalling a low level of aggression due to its higher aggressiveness but would not engage in escalated fighting with individuals honestly signalling high aggression. Unless individuals were forced to pay a contest-independent cost equivalent to their aggressiveness, Trojans would therefore invade by out-competing honest low-aggression individuals (Owens & Hartley 1991; Johnstone & Norris 1993). However, this conclusion has been criticised by Maynard Smith & Harper (2003) and Hurd (1997), while Számadó (2011) has argued that commitment to defending a resource over a long period of time can maintain honesty in status signals without the need for additional costs incurred outside of fights.

Given that contest-independent costs may be important in maintaining honesty in status signals, potential costs associated with high aggression and/or RHP have been investigated (reviewed in Senar 1999). Some studies have argued that aggressive individuals pay costs associated with supporting an increased metabolic rate (e.g. Järvi & Bakken 1984), while others have suggested that the high hormone titres that underpin aggressive behaviour may impose physiological costs, including suppression of the immune system (e.g. Evans *et al*. 2000; Tibbetts & Banan 2010). While costs associated with high metabolic rates and/or high hormone titres may be sufficient to deter cheating, empirical evidence for this idea is mixed (Senar 1999; Roberts *et al*. 2004). Another suggestion for a contest-independent cost is the higher risk of predation faced by individuals signalling a high status (e.g. Slotow & Rothstein 1995). However, evidence

for this idea is also limited (Senar 2006). Moreover, if predation risk increases as a consequence of signal intensity, this would suggest that the signal itself imposes the cost, which is more consistent with strategic signalling than with status signalling (though see e.g. Guilford & Dawkins 1995, who see no reason why contest signals cannot have both receiver-dependent and independent costs). Finally, researchers have suggested that status signals may be honest because they are also targets of female choice (that is, they are under inter- as well as intrasexual selection). However, while male status signals do appear to function in female choice in a number of species (e.g. house sparrows (*Passer domesticus*): Møller 1988; collared flycatchers: Gustafsson *et al*. 1995; Qvarnström 1997), it remains unclear whether intersexual selection can be successful in stabilising status signalling, or whether honest status signalling is a necessary condition for the secondary evolution of status signals as targets of female choice (Berglund *et al*. 1996).

Summary

Since its inception in the 1970s with the studies of Rohwer and colleagues, research on the use of status signals in animal contests has been hampered by controversy and confusion (e.g. Shields 1977; Guilford & Dawkins 1995; Senar 1999; Maynard Smith & Harper 2003). In a number of species, patches of colour are found to predict an individual's aggression or RHP and manipulative experiments have indicated that this coloration is used to signal information about agonistic ability to rivals during contests (Senar 1999, 2006; Whiting *et al*. 2003). The fundamental controversy over status signals has centred on the problem of how these signals can be honest (Maynard Smith & Harper 2003). In the apparent absence of significant production costs, explanations for signal honesty have focused on social costs, which are argued to arise from aggressive interactions with receivers (Rohwer 1977).

However, the idea that social costs can stabilise signal honesty is problematic, for a number of reasons. First, clear evidence for a mechanism by which dishonest signallers are forced to incur social costs is lacking (Senar 1999; but see Számadó 2011). In particular, there is limited evidence for social costs based on like versus like aggression, as predicted by the social control hypothesis, or for receiver retaliation based on

perceived incongruities between the signal and behavioural cues, as predicted by the incongruence hypothesis (Senar 1999; but see Tibbetts & Dale 2004; Tibbetts & Izzo 2010). Second, the reliance on social costs to stabilise status signals appears paradoxical, as it seems to require that receivers believe the information sent by the signaller in some contexts, but while aggressively interrogate this information in others (Senar 1999). Third, as noted above, it is unclear whether social costs are sufficient to prevent cheating, or whether contest-independent costs are required (Johnstone & Norris 1993). Several such costs have been proposed, including physiological costs associated with aggression and RHP and costs exerted via mate choice, which could potentially stabilise status signals (Järvi & Bakken 1984; Evans *et al.* 2000). However, in the search for contest-independent costs to stabilise status signals, the distinction between status signals and other forms of contest signal (e.g. indices and handicaps) has become increasingly blurred, for example, where status signals themselves exert a cost by increasing the risk of predation (Slotow & Rothstein 1995) or where the expression of the signal is causally linked to behaviour via shared hormonal pathways (Ducrest *et al*. 2008; see also Chapter 8).

The existence of cheap, arbitrary signals of agonistic ability has thus presented special difficulties to those researchers seeking to understand the evolution and maintenance of honest signalling systems. In the last decade, however, a series of studies of status signalling in the paper wasp *Polistes dominulus* have been argued to provide resolutions to many of these difficulties and, in doing so, have helped to revive the concept of status signalling, particularly within social insects.

Status signalling in *P. dominulus* is the subject of the first part of this thesis (Chapters 3-5). Below, I provide an brief introduction to *P. dominulus*, including an overview of the important aspects of its biology. I then move to review the evidence for and against status signalling in *P. dominulus*, before outlining the research questions to be addressed in Chapters 3-5.

1.2.3 An introduction to *Polistes dominulus*

Polistes is a species-rich genus of social wasps within the vespid subfamily Polistinae (Carpenter 1991). The genus comprises 204 species, including three obligate social parasites (Arévalo *et al*. 2004). The genus has a world-wide distribution, though the majority of species are found in the tropics (Carpenter 1991). *Polistes* exhibits independent colony founding, in which small paper nests are constructed either by a single mated female (foundress) or by a small number of co-foundresses (Reeve 1991). *Polistes* displays a degree of cooperative behaviour termed 'primitive eusociality' (Reeve 1991). Thus, within groups, there is often a pronounced division of labour and reproduction between the dominant foundress and her subordinate helpers, yet these helpers (which include both subordinate co-foundresses and the dominant's offspring) are not sterile as in advanced eusocial species, but are capable of independent reproduction (Reeve 1991). The behavioural flexibility exhibited by females in many species have contributed to the use of *Polistes* wasps as model organisms for the study of social evolution, in particular the evolution of cooperative behaviours (e.g. Field *et al*. 1998; Reeve *et al*. 2000; Shreeves *et al*. 2003; Leadbeater *et al*. 2010, 2011).

Polistes dominulus[2] (Christ, 1791) is among the best studied of all primitively eusocial wasps. A temperate species, *P. dominulus* has a widespread and cosmopolitan distribution, following recent expansions from its native Afro-Eurasian range into North and South America and Australia (Cervo *et al*. 2000). The colony cycle of *P. dominulus* is typical of temperate *Polistes* species (Reeve 1991). In spring, nests are founded by a single (lone) foundress or by small groups of co-foundresses (usually <10) (Figure 1.1). In co-foundress groups, a dominance hierarchy emerges following a period of intense aggression between foundresses at the start of nest founding, which includes prolonged physical combat and 'falling fights', where individuals fall through the air grappling and attempting to sting one another (Pardi 1948; Figure 1.1). With the emergence of the hierarchy, fighting subsides, and is largely replaced by ritualised dominant-subordinate

[2]The correct nomenclature is *Polistes dominula*. As Buck *et al*. (2008) note, *dominula* is the diminutive form of the Latin noun *domina* ('mistress') and as such is indeclinable. However, *dominulus* continues to be widely used in the literature; for reasons of consistency, I therefore refer to *P. dominulus* in this thesis.

behaviours, the most conspicuous of which is ‘mounting’, whereby a subordinate lowers its antennae and permits a dominant to stand on its head (Röseler 1991).

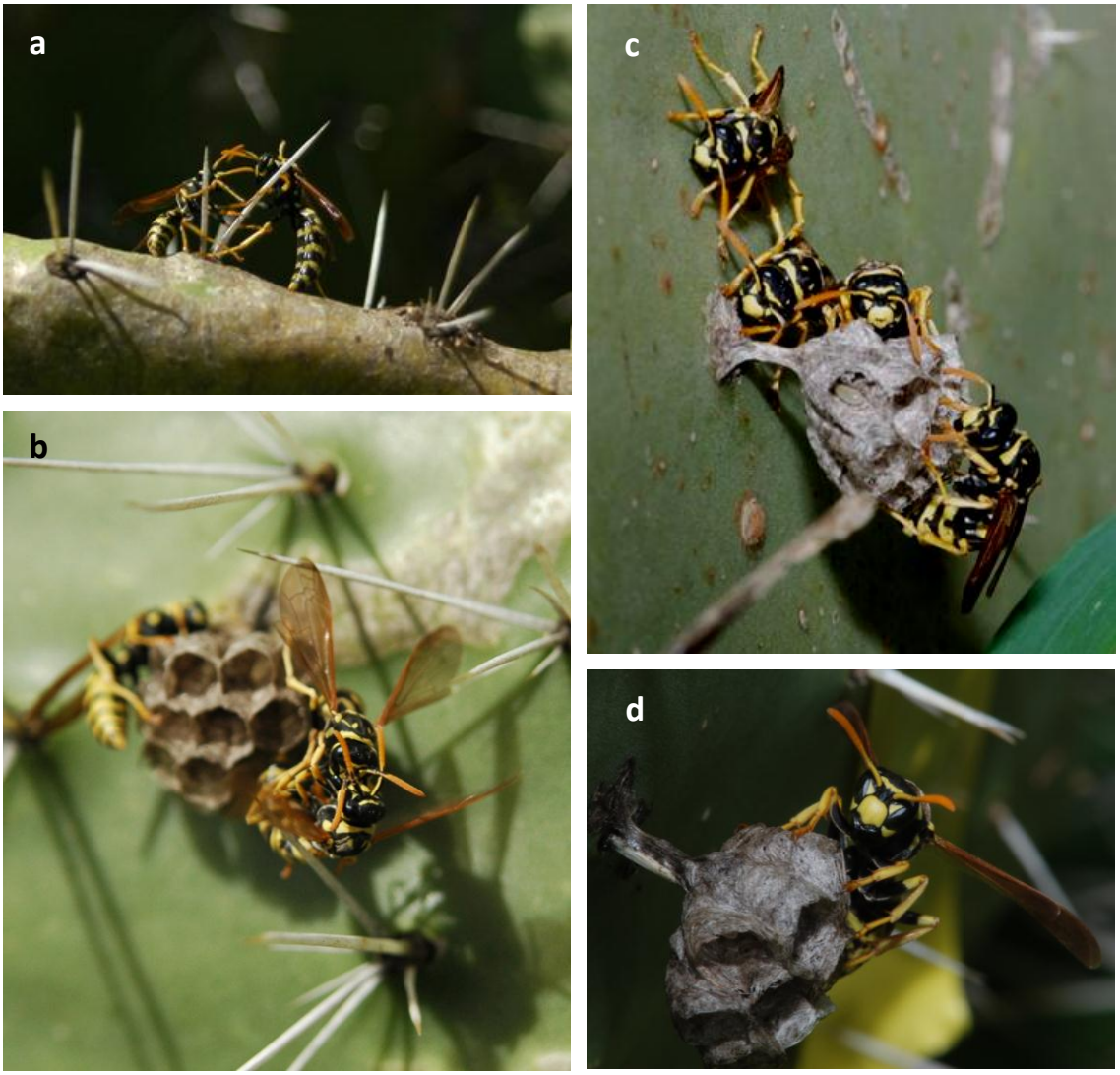


Figure 1.1 *P. dominulus* foundresses during the nest-founding phase. (a) The nest-founding phase is characterised by high levels of aggression between foundresses. Here, two foundresses are seen fighting away from the nest. (b) Fighting may escalate, leading to ‘falling fights’. In the spring, nests may be founded by groups of co-foundresses (c) or else by a single (lone) foundress (d). Photographs (a) and (b) by Jasper van Heusden.

The hierarchy plays a critical role in determining the distribution of group benefits among co-foundresses. First and foremost, rank within the hierarchy determines access to reproduction, with the dominant individual producing the majority of offspring at any one time (Pardi 1948). However, an individual's rank also affects its future reproductive prospects, with those at higher ranks more likely to inherit the dominant position in the event that it becomes vacant (Leadbeater *et al*. 2011). The hierarchy also has an important influence on the division of labour within groups. Subordinates typically undertake frequent foraging trips to collect food (mostly Lepidopteran larvae) for the developing offspring, as well as water and nest-building materials, while the dominant foundress forages only infrequently and instead remains on the nest feeding offspring and engaging in nest construction (Röseler 1991). Among subordinates, however, the amount of effort invested in foraging varies with their position in the hierarchy, with those at higher ranks (and therefore near the front of the breeding 'queue') undertaking less foraging than individuals at lower ranks (Cant & Field 2001). Finally, hierarchical rank also determines the share of food that an individual receives, with food-sharing and trophollaxis preferentially directed towards individuals of higher rank (Röseler 1991).

The nest-founding phase ends with the eclosion of the first offspring. A high proportion of these early offspring are female. The majority of these females remain on the natal nest as workers, undertaking activities such as foraging and nest defence, though a small proportion are thought to leave to pursue independent reproduction (Reeve 1991). During the worker phase, the numbers of subordinate co-foundresses on nests is observed to fall, possibly as a result of eviction by the dominant who is now able to rely on the workers for help. At the same time, rates of dominant replacement increase, with co-foundresses originally occupying subordinate positions now assuming the dominant position (Leadbeater *et al*. 2011). Workers, who upon eclosion are thought to join at the bottom of the hierarchy, are observed to inherit the dominant position only following the death or disappearance of all the original co-foundresses (Monnin *et al*. 2009).

Towards the end of the season in mid to late summer, colonies pass from the worker phase into the reproductive phase. At this time, greater numbers of male offspring eclose on the nest, while eclosing females remain on the nest and do not participate in foraging or other cooperative behaviours (Reeve 1991). Gradually, both male and

female offspring disperse, at which point the colonies dissolve. Mating then occurs, after which males die. In the autumn, mated females aggregate in shelters where they spend the winter in diapause before emerging to found nests in the spring (Reeve 1991).

P. dominulus has long been a focus of research into a broad range of topics within evolution, behaviour and ecology, including social evolution (e.g. Shreeves *et al*. 2003; Liebert & Starks 2006; Leadbeater *et al*. 2010, 2011), host-parasite co-evolution (e.g. Ortolani & Cervo 2009, 2010), invasion biology (e.g. Cervo *et al*. 2000; Liebert *et al*. 2006) and communication (Tibbetts & Dale 2004; Dapporto *et al*. 2007). Until recently, research on communication in *P. dominulus* and other social wasps has focused exclusively on chemical compounds (chiefly hydrocarbons) on the surface cuticle, which have been implicated in various forms of recognition, including recognition of nest-mates and potentially also recognition of a nest-mate's rank in the hierarchy (Sledge *et al*. 2001; Gamboa 2004; Dapporto *et al*. 2007). In the last decade, however, *P. dominulus* has become the focus of research into a hitherto unexplored mode of communication in social insects, and one which has attracted considerable excitement and controversy. In the following section, I review this research and consider the evidence for visual signals of status in *P. dominulus*.

1.2.4 Visual signals of status in *P. dominulus*

Background

During the Greek and early Roman empires, soldiers in battle fought with circular shields known as *clipei* (Smith *et al*. 1890; Figure 1.2a). Primarily for defensive use, these shields were often richly ornamented with symbols of a soldier's pride, spirit, ancestry or else scenes from mythology. In several cases, however, the design of the *clipeus* was decidedly more threatening, with some carrying metal bosses designed to inflict injury, while others presented terrifying images, possibly in an attempt to frighten enemy combatants (Smith *et al*. 1890). A notable example of the latter is the *clipeus* of Agamemnon, commander of the Greeks during the Trojan war, which carried images of the Gorgon, and of Deimos and Phobos, the gods of dread and fear (Homer, *Iliad*, 11.36, in Smith *et al*. 1890).

Although the *clipeus* has long since disappeared from human warfare, the word survives today (as 'clypeus') and is used instead to refer to an area on the front of an insect's head between the labrum and the frons (Figure 1.2b). Among social wasps, there is great variety in the patterns of coloration found on the clypeus. Variation in clypeal patterning among species is routinely used as a character in species identification (e.g. Buck *et al*. 2008); however, variation in patterning within species has received little attention (but see Enteman 1904). In *P. dominulus*, clypeal patterning is highly variable in females, with individuals presenting either a completely yellow clypeus, or else clypeal patterns consisting of black spots of various shapes and sizes. Far from being mere ornamentation, however, recent studies by Tibbetts and colleagues indicate that these patterns are more akin to the symbols of power and terror that adorned the original clipei of the Greek and Roman armies. In particular, the clypeal patterns are argued to function as signals of status, which are used during conflict to communicate RHP to rivals, thereby allowing the likely winner to be determined and the dispute to be settled before it escalates into dangerous fighting (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts & Shorter 2009; Tibbetts & Izzo 2010; Tibbetts *et al*. 2010).

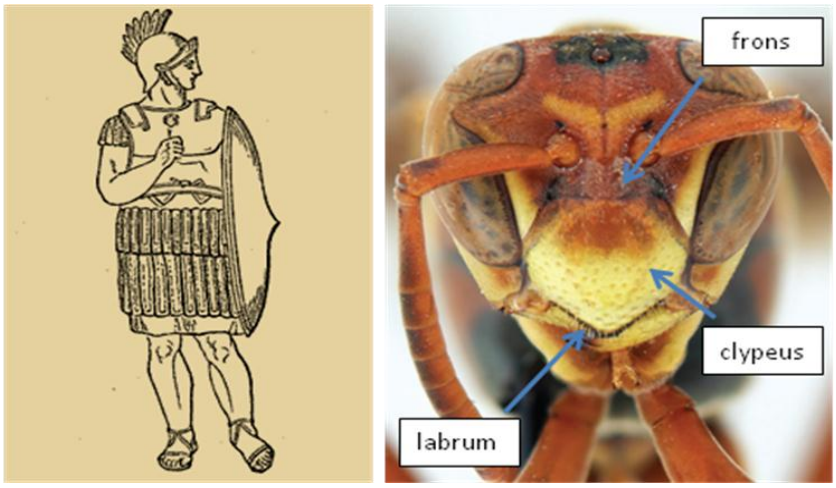


Figure 1.2 (a) Illustration of a Roman soldier bearing a *clipeus* from the column of Trajan in Rome (image from Smith *et al*. 1890). (b) Front view of the head of a wasp (*Polistes bellicosus*) with arrows showing the position of the clypeus, labrum and frons (photograph from Buck *et al*. 2008).

Prior to the publication of the study by Tibbetts & Dale (2004), evidence of visual status signals in insects came from a single study by Beani & Turillazzi (1996), who showed that males of the stenogastrine wasp *Parischnogaster mellyi* display white abdominal stripes during mating patrols, and that artificial enhancement of these stripes led to increased aggression from other males (Beani & Turillazzi 1996). In *P. dominulus*, in contrast, it is the females that are argued to use the status signal, based on variable black patterns on the clypeus. The aspect of the pattern that is argued to communicate RHP is its 'brokenness' (Tibbetts 2010), which provides a measure of the amount of disruption in the pattern. The method for scoring patterns using the brokenness index of Tibbetts (2010) is outlined in Chapter 2. In brief, individuals without black patterns (i.e. those with a yellow clypeus) have a brokenness score of zero. For individuals with one or more black spots on the clypeus, brokenness is a function of the number, size and shape of the spots. Individuals with more spots tend to have higher brokenness; however, among individuals with the same number of spots, those with more disrupted spots will tend to have higher brokenness than those with more uniform spots (Figure 1.3).

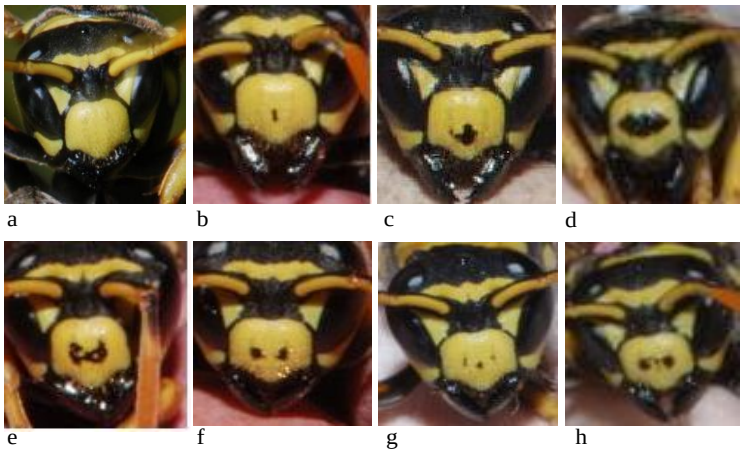


Figure 1.3 Portraits of eight *P. dominulus* females collected in southern Spain in 2008-09, arranged in order of increasing brokenness of the clypeal pattern. Brokenness increases with the number of clypeal spots from 0 spots (a), through 1 spot (b-e), 2 spots (f) and 3 spots (g-h). Brokenness also increases with the size of the spots. Thus, wasp *d* has a higher brokenness than wasps *b-c*, and wasp h has a higher brokenness than wasp *g*. Finally, brokenness increases with the amount of disruption in the pattern. Wasp *e* thus has a higher brokenness than wasp *d*, though both have a single spot of similar size.

Experimental demonstrations of status signalling

To date, evidence that brokenness signals RHP to rivals comes from studies by Tibbetts and colleagues on two populations from the northeastern United States[3]. In the first study, Tibbetts & Dale (2004) found a positive correlation between brokenness and body size, which some studies indicate may be an important determinant of RHP in *P. dominulus* (e.g. Turillazzi & Pardi 1977; but see Zanette & Field 2009). Furthermore, the authors showed that brokenness predicted the outcome of lab-based dominance interactions, as judged by the rates of mounting behaviour. Finally, in a second round of dyadic interactions, one individual was manipulated with paint to increase or decrease the brokenness of its pattern. This manipulation had no effect on which individual initially mounted the other (i.e. which individual assumed dominance); however, subsequent rates of mounting by the dominant were higher when the subordinate had experimentally-altered clypeal patterns, and were highest when the subordinate was painted to have an artificially high level of brokenness. While manipulation of the pattern did not affect the outcome of the dominance interaction, Tibbetts & Dale (2004) argued that brokenness is a signal of status, based on the observation that manipulated individuals receive more aggression following dominance establishment. They interpret this pattern of behaviour as rare support for the existence of social costs acting to stabilise signal honesty by inflicting punishment on individuals bearing dishonestly high (or low) levels of brokenness (Tibbetts & Dale 2004).

However, although the aggressive responses to the manipulations appear consistent with the infliction of social costs, the study actually provides no direct evidence that the clypeal pattern (and in particular the brokenness of the pattern) functions as a status signal. Indeed, as Strassmann (2004) notes, the fact that individuals painted to have a high brokenness fail to achieve dominance during agonistic interactions appears to argue against brokenness as a signal of status. In contrast, Tibbetts & Dale (2004) argue that this failure is to be expected, given the existence of social costs that act to punish individuals whose clypeal patterns do not accurately reflect underlying RHP (Rohwer & Rohwer 1978). However, in advancing this argument, Tibbetts & Dale (2004) appear to imply that the failure to demonstrate a signalling function in the clypeal patterns is a

[3] A summary of the research on status signalling in *P. dominulus* discussed in this section is provided in Appendix A.

result of social costs acting to enforce the honesty of the signal. In other words, it appears impossible to test for a signalling function without accidentally triggering an anti-cheating mechanism, which obscures the effect of the signal manipulation (Senar 1999). Somewhat worryingly, therefore, merely failing to demonstrate status signalling would not appear to be sufficient grounds for rejecting the hypothesis that signalling is occurring, in the event that social costs might be operating.

One solution to this problem has been to test receiver responses to pattern manipulations on dead animals, thereby disentangling an individual wasp's behaviour from its advertised brokenness. In experiments similar to those of Senar & Camerino (1998) (see above), Tibbetts & Lindsay (2008) gave wasps the choice between two food sources (sugar cubes). On top of each cube was positioned a dead wasp ('guard'). Each guard in a pair was painted to alter the brokenness of the clypeal pattern, resulting in pairs comprising a 'high-spot' and a 'low-spot' guard (in these studies, the number of clypeal spots was used as a proxy for brokenness). Using this approach, the authors found that wasps preferred to eat at the cube near to the low-spot guard. In a later experiment, Tibbetts *et al*. (2010) found a similar result, except that the likelihood of choosing the low-spot guard was influenced by the focal wasp's advertised brokenness. These experiments provide an arguably more satisfying demonstration of status signalling than in Tibbetts & Dale (2004), as they show that individuals respond to variation in clypeal patterning alone, and that these responses are consistent with assessment of rival RHP. Moreover, such behavioural responses occur in an obvious competitive context with a clearly-defined resource, and in the case of Tibbetts *et al*. (2010), appear to point to a process of 'mutual assessment', whereby individuals incorporate information about their own RHP into their assessment of fight outcome (Arnott & Elwood 2008).

However, it is unclear from these results why individuals should choose to attend to a rival's advertised brokenness during some encounters (e.g. disputes over sugar), while seeming to ignore it in other contexts (e.g. the encounters staged by Tibbetts & Dale [2004]). An elegant solution to this problem was provided by Tibbetts (2008), who showed that the likelihood of a wasp approaching food guarded by a high-spot guard increased with its level of hunger, whereas hunger levels did not influence the decision to approach the low spot guard. This result was interpreted as evidence for context-

dependence in the response of receivers to the brokenness signal, with responses to the signal dependent on the motivation of the receiver during the contest (Tibbetts 2008). Thus, when receivers perceive the value of the contested resource to be high – for example, following starvation (Tibbetts 2008) or during dominance establishment (Tibbetts & Dale 2004) – rival assessment is abandoned in favour of escalation in a bid to secure the resource.

Tibbetts' (2008) result is important for two reasons. First, it provides empirical support for two predictions made by theoretical analyses of contest behaviour, namely that rival assessment based on status signals is not expected where resource value is high (Maynard Smith & Harper 1988) and that escalation is favoured where the cost of failing to acquire the resource exceeds the cost of escalated fighting (Enquist & Leimar 1987). Moreover, this result offers a resolution to a paradox that appears at the heart of status signalling. As discussed above, status signalling permits assessment of rival RHP, which is argued to reduce the costs of escalated conflict. Yet the stability of status signals seems to require that the receiver aggressively tests the signaller to determine its true RHP. Both are feasible, however, if receiver responses are context-dependent. Where the resource is of low value, individuals may accept the level of RHP signalled by a rival; however, when resource value is high, individuals are motivated to fight, with the resulting escalation functioning as a test of signal accuracy (Tibbetts 2008).

The relationship between brokenness and RHP

In parallel with research into the signal value of clypeal patterns, other studies have investigated the relationship between brokenness and aspects of quality, including RHP (Tibbetts 2006; Tibbetts & Curtis 2007; Tibbetts & Izzo 2009, 2010; Tibbetts & Shorter 2009; Tibbetts 2010; Tibbetts & Banan 2010; Tibbetts *et al*. 2011a,b). Several of these studies provide further evidence of a positive correlation between brokenness and RHP. Tibbetts & Shorter (2009) found that asymmetries in brokenness predicted the winner of staged nest usurpation contests. In a second study, Tibbetts *et al*. (2011a) found that brokenness predicted the outcome of dyadic contests, similar to those used in Tibbetts & Dale (2004). However, in this study, a positive effect of brokenness on contest outcome was observed in only one of the two years in which contests were staged.

Research has also focused on identifying the causal link between brokenness and RHP. Examining the clypeal patterning of offspring eclosing over the season, Tibbetts (2006) found that offspring emerging late in the season had more broken patterns and argued that this trend reflects the higher quality of late-emerging offspring, which receive more food during development. To test whether brokenness is sensitive to nutritional status, Tibbetts & Curtis (2007) compared clypeal patterning between offspring emerging from colonies that had been provided with additional food and colonies that had not received extra food. The authors found that brokenness was higher when offspring received more food, indicating that brokenness varies with nutritional status. This result was later replicated by Tibbetts (2010), who demonstrated a heritable component to brokenness, but showed that this heritability was lower among individuals from food-supplemented colonies. Together, these findings have been interpreted as evidence that brokenness signals aspects of individual quality, and that its expression is condition-dependent and varies with environmental conditions (Tibbetts & Curtis 2007; Tibbetts 2010).

While these studies indicate that brokenness may reflect aspects of individual quality, others have delved further in an effort to reveal the causal link between brokenness and RHP. A primary target of this research has been juvenile hormone (JH), a major insect hormone that affects many aspects of behaviour and physiology, including fertility and dominance behaviours (Tibbetts *et al*. 2011a). Several lines of evidence suggest that JH may mediate the link between brokenness and RHP. First, brokenness is positively correlated (albeit weakly) with natural JH titres (Tibbetts *et al*. 2011a). Second, brokenness appears to predict an individual's ability to withstand artificial increases in JH titres (Tibbetts & Izzo 2009; Tibbetts & Banan 2010). Indirect evidence also comes from a study by Tibbetts *et al*. (2011b), who found a correlation between increased brokenness and earlier time of emergence from diapause. Tibbetts *et al*. (2011b) have argued that this result can be explained by the enhanced condition of wasps with more broken patterns, which allows them to become active at lower temperatures. Furthermore, as JH titres rise rapidly following emergence from diapauses (Röseler 1991), the authors suggest that the difference in emergence time may explain the higher titres of JH in more broken individuals (Tibbetts *et al*. 2011a).

Based on these findings, Tibbetts *et al*. have proposed that brokenness may provide information about an individual's RHP by signalling its ability to bear the cost of JH (Tibbetts & Banan 2010; Tibbetts *et al*. 2011a). Under this scenario, only high-quality individuals (i.e. those enjoying high levels of nourishment during development) are able to support high levels of JH production and circulation, which is then reflected in the high level of brokenness in these individuals' clypeal patterns. Furthermore, it has been suggested that the condition-dependent costs of JH can act to stabilise the brokenness signal and prevent dishonest signalling by low-quality individuals, who are unable to support the high costs of JH (Tibbetts & Banan 2010).

What then of the social costs argued to stabilise the brokenness signal by Tibbetts & Dale (2004)? In a recent experiment, Tibbetts & Izzo (2010) revisited the idea of social costs as a further means of ensuring signal honesty. Following the methods of Rohwer & Rohwer (1978) and Järvi *et al*. (1987), Tibbetts & Izzo (2010) used a combination of paint manipulations and JH application to create 'cheats' with either high brokenness and reduced aggression or low brokenness and heightened aggression. The authors found that such individuals incurred greater costs during dyadic contests, which they interpreted as support for the incongruence hypothesis (Rohwer & Rohwer 1978), whereby dishonest signallers incur costs in response to a perceived incongruity between the level of RHP signalled and its behaviour (Tibbetts & Izzo 2010). Thus, in *P. dominulus*, reliability of the signal appears to be based on both contest-dependent (i.e. social) and contest-independent costs (possibly through an interaction between quality and JH) (Tibbetts & Izzo 2009, 2010; Tibbetts & Banan 2010; Tibbetts *et al*. 2011a).

In summary, studies by Tibbetts and colleagues on North American populations of *P. dominulus* have provided evidence of a status signal based on the amount of disruption (brokenness) in the clypeal pattern. This signal appears to be important in rival assessment during competition (Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010) and asymmetries in brokenness have been shown to predict the outcome of agonistic interactions (Tibbetts & Dale 2004; Tibbetts *et al*. 2011a). Furthermore, both condition dependence and social costs appear to be important in stabilising the signal, such that brokenness reliably reflects underlying RHP (Tibbetts & Dale 2004; Tibbetts & Curtis 2007; Tibbetts 2010; Tibbetts & Izzo 2010; Tibbetts *et al*. 2011a). In spite of these

findings, however, the empirical support for status signalling remains far from clear-cut. Below, I describe a number of difficulties arising from studies of status signalling, both in North America and elsewhere.

1.2.5 Status signalling in *P. dominulus*: outstanding difficulties

Relationship between brokenness and RHP

In the study by Tibbetts & Dale (2004), brokenness was argued to reflect RHP based on a positive correlation between brokenness and body size. However, this correlation was very weak, with brokenness accounting for only a small amount of the variance in size. Moreover, in a subsequent experiment, Tibbetts & Curtis (2007) found that offspring receiving more food developed more broken patterns but were smaller in size. Brokenness would therefore appear to be, at best, an unreliable indicator of size. Independent of size, brokenness was found to predict the outcome of agonistic interactions in three studies (Tibbetts & Dale 2004; Tibbetts & Shorter 2009; Tibbetts *et al*. 2011a). In the latter study, however, brokenness predicted the winner in only one year out of two (Tibbetts *et al*. 2011a). The reason for this variation is not clear, but would appear to indicate that the relationship between RHP and brokenness is not always positive, even within the same population.

Dominance interactions in the lab

An important question in any study of signal function is whether the behavioural criteria used to assess receiver responses are appropriate. Evidence for social costs in the studies by Tibbetts & Dale (2004) and Tibbetts & Izzo (2010) came from observations of what were termed ‘dominance interactions’ between individuals in the lab, in particular, the frequency of mounting behaviour. There are at least three potential problems with this approach. First, dominance interactions of the kind observed in the lab differ markedly from those in the wild. In the wild, dominance hierarchies arise during periods of intense competition between foundresses over ownership of nests and reproduction within groups (Reeve 1991). In contrast, dominance interactions in the lab take place in a neutral arena in the absence of any obvious resource, which raises questions about the motivation of individuals to fight during interactions in the lab. Second, the two-hour

period in which individuals were allowed to interact in the lab may be insufficient for individuals to develop meaningful and lasting dominance relationships (Cervo *et al*. 2008). For these reasons, it is possible that interactions in the lab may not be a reliable guide to dominance relationships, and that the outcome of such interactions may not accurately reflect asymmetries in RHP between individuals.

A final concern is the strong emphasis that is placed on mounting behaviours as a guide to dominance relationships and as a measure of social costs. Observations of behaviour in the wild indicate that mounting is a ritualised dominance behaviour, which functions to reinforce dominance relationships following hierarchy establishment (Röseler 1991). Like other ritualised behaviours, mounting is thought to be energetically inexpensive and carry little risk of injury (Röseler 1991). It is therefore unclear why Tibbetts & Dale (2004) base their evidence for social costs on rates of mounting, when such costs are typically thought to arise from escalated fighting and aggressive testing of the receiver (Tibbetts 2008). Moreover, the use of mounting as a guide to dominance may produce misleading results in the event that individuals do not establish proper dominance hierarchies during the short period of interaction in the lab (Cervo *et al*. 2008).

The importance of clypeal patterning in the wild

Arguably the biggest difficulty with the hypothesis that clypeal patterns function as a signal of status is the absence of data from wild populations showing that individuals with high brokenness enjoy a fitness advantage over those with less broken patterns. To date, all the studies purporting to demonstrate status signalling and a link between brokenness and quality have been carried out in the lab using individuals from only a few American populations, with the exception of Tibbetts (2006) and Tibbetts (2007), who recorded the clypeal patterning of wasps in the wild. Surprisingly, there have been no detailed studies of how brokenness varies with RHP or other aspects of quality in these populations. Moreover, very little information is available about the populations, in terms of foundress nesting strategies and the abundance of resources, both of which would likely influence the degree of competition between individuals, and therefore the pay-offs associated with rival assessment.

However, though the importance of brokenness in the wild North American populations is currently unknown, several studies have explored clypeal patterning in wild populations in *P. dominulus*' native European range. The most detailed of these studies, found no correlation between pattern brokenness and hierarchical rank in co-foundress groups transferred to the lab from sites in central Italy (Cervo *et al*. 2008). Furthermore, brokenness did not predict survival over winter or parasitism by strepsipteran endoparasites (Cervo *et al*. 2008). Of these findings, the absence of a correlation between brokenness and hierarchical rank seems the most important, as competition between females over rank is well described in the wild (e.g. Pardi 1948). Even in the event that status signals are not used during contests over rank (for instance, if the high value of the social rank favours escalation over assessment), a positive correlation between rank and brokenness would nonetheless be expected if brokenness reflected individual RHP (Cervo *et al*. 2008). The absence of a correlation between brokenness and rank therefore indicates that brokenness does not reflect RHP in this population (although, as noted above, colonies were transferred to the lab prior to behavioural observations, which may have disturbed natural social interactions).

In a second study, Zanette & Field (2009) looked for correlations between rank and clypeal patterning within wild-nesting co-foundress groups in a population in southern Spain. A weak positive correlation between clypeal patterning and rank among the top-ranking foundresses was observed; however, the effect of clypeal patterning on rank was not significant after controlling statistically for other predictors of rank. While the authors argue that this result is consistent with the idea that clypeal patterns function as signals of quality, it is important to note that the study did not look at brokenness, but instead measured the size of the clypeal pattern. It therefore remains unclear whether brokenness predicts RHP among foundresses in the Spanish populations.

Finally, clypeal patterning has been studied in several populations in Ukraine by Rusina and colleagues. Again, these studies did not attempt to quantify brokenness, but looked at the size of the clypeal spots. Rusina *et al*. (2006) found that foundresses attempting to usurp single-foundress nests tended to have larger spots than the resident foundress, and that clypeal patterning predicted the outcome of usurpation contests. However, in a later study, Rusina *et al*. (2007) found that clypeal patterning did not predict hierarchical

rank in co-foundress groups, which appears to contradict the previous result and argue against a relationship between patterning and RHP in this population.

Overall, therefore, there is little evidence of a positive relationship between brokenness and RHP in Europe. However, only the study by Cervo *et al*. (2008) explicitly looked at the association between brokenness and hierarchical rank, whereas the relationship between brokenness and rank was not investigated in the Spanish or Ukrainian populations. Moreover, while hierarchy establishment is thought to be an important focus for competition between foundresses (Reeve 1991; Röseler 1991), there are other contexts in which brokenness may be important, including contests over food (Dapporto *et al*. 2005) and nest ownership (Tibbetts & Shorter 2009).

Discussing their results, Cervo *et al*. (2008) highlight possible differences between the American and European populations that could account for the conflicting findings. One striking difference is the degree of variability in clypeal patterning. In North America, a high proportion (80%) of wasps have some degree of clypeal patterning (Tibbetts & Dale 2004), but in Italy this proportion is only 40% (Cervo *et al*. 2008), while in Spain it is as low as 15% (Zanette 2007). Whether this points to a genuine difference between populations in the adaptive value of patterns, however, remains unclear. Zanette & Field (2009) interpret the low variability in patterning as evidence that brokenness can be neither sufficient nor necessary for the attainment of high rank, but could still signal RHP. However, Cervo *et al*. (2008) view the low variability in patterning in their population as strong evidence against the use of clypeal patterns as status signals. Cervo *et al*. (2008) also reject the idea that the signal value of clypeal patterns may vary between populations. In particular, the authors argue that the lack of a relationship between brokenness and RHP in Europe (the species' native range) is more likely to be 'biologically real' than the results obtained from the recently-founded North American populations, and that there have been too few generations in the American populations to permit the evolution of signalling there (Cervo *et al*. 2008).

However, given the important gaps in research in both the European and American populations, I would argue that Cervo *et al*.'s strong rejection of status signalling in *P.*

dominulus is both untimely and overly simplistic. Rather, I believe that research into status signalling in this species would benefit considerably from answers to the following three questions. First, do clypeal patterns signal status in European populations? Surprisingly, there has to date been no test of signal function in European populations. Despite evidence that clypeal patterning is not correlated with RHP (Cervo *et al*. 2008), an experiment is needed to determine whether clypeal patterns function in rival assessment in Europe. Second, how does clypeal patterning relate to fitness in the wild? Despite previous efforts to address this question (Cervo *et al*. 2008; Zanette & Field 2009), the relationship between brokenness and aspects of quality, including RHP, remains poorly understood. Establishing how brokenness relates to fitness is important if we are to understand the adaptive value of clypeal patterning in European populations. Third, what are the underlying causes of the differences in pattern variability between populations? This question has received no attention to date, but may help to explain any difference in the signal value of clypeal patterns between populations.

1.2.6 Part I research aims

In Part I of this thesis (Chapters 3-5) I explore the three questions above. In Chapter 3, I undertake an experimental test of clypeal pattern signal function in the Spanish population studied by Zanette & Field (2009), following the method of Tibbetts & Lindsay (2008). In Chapter 4, I then move to explore the relationship between clypeal patterning and aspects of fitness among free-living wasps in the Spanish population. Finally, in Chapter 5, I explore the factors responsible for variation in clypeal pattern expression between populations.

1.3 Information gathering during host-parasite interactions

Above, I have considered information gathering in conflict between conspecifics. However, conflict over resources also occurs between members of different species, and in particular is a defining characteristic of host-parasite interactions (Sorci & Cézilly 2008). In this section, I will explore how information is used in conflicts between a paper wasp and its brood parasite. Below, I briefly outline the importance of information gathering in host-parasite interactions. I then provide an overview of brood parasitism, before describing my study system in more detail.

In heterospecific host-parasite associations, conflict over resources can occur at several levels. In many associations, the parasite feeds directly on the host (i.e. the host is the resource). In others, however, the parasite may exploit a host's food supply (kleptoparasitism) or host parental care (brood parasitism) (Sorci & Cézilly 2008). Parasitism often imposes significant fitness costs on the host, which are expected to exert strong selection for host defences against parasitism. Similarly, the dependence of many parasites on their hosts for survival and reproduction is expected to favour the evolution of mechanisms to overcome host defences. Together, these opposing selection pressures can result in a co-evolutionary 'arms-race' between hosts and parasites, where host adaptations against parasitism favour the evolution of counter-adaptations in the parasite (Toft & Karter 1990; Rothstein 1990).

Information gathering plays an important role in the evolution of both host adaptations against parasitism and parasite counter-adaptations. In general, parasites need to be able to identify suitable hosts, and to gain access to host resources without being detected, whereas hosts need to be able to detect parasites and take the appropriate steps to minimise their impact (Bush *et al*. 2001). Where parasites live in or on the host and feed on its tissues, hosts can gather information about parasites through the actions of the immune system, which in the first instance functions to detect the presence of parasites and other pathogens via molecular cues (Bush *et al.* 2001). Alternatively, where parasites exploit resources outside the host's body, as in the case of kleptoparasitism or brood parasitism, hosts can potentially acquire information about parasites from cues in

the external environment (including from the parasite itself), which are detected by the host's sensory systems (Lenoir *et al*. 2001; Lorenzi 2006). In this thesis, I focus on the role of information gathering in mediating conflicts between brood parasites and their hosts. Below, I provide a short introduction to brood parasitism, focusing on the opportunities for information gathering in host-brood parasite associations.

1.3.1 Brood parasitism

Brood parasites manipulate host parental care, redirecting parental effort and resources towards parasite young (Cervo 2006). Brood parasitism may be interspecific, where one species parasitises a second, or intraspecific, where conspecifics parasitise one another. Interspecific brood parasitism is observed in a number of taxa, notably birds (Rothstein 1990) and insects (D'Ettorre & Heinze 2001; Cervo 2006). In all cases, the parasite relinquishes the care of its brood to another (heterospecific) individual. In order to achieve this, the parasite must place its offspring within the nest of a host[4]. For avian brood parasites (e.g. cuckoos and cowbirds), this is a very rapid process, and does not usually involve any contact between the parasite and adult hosts (Rothstein 1990). In contrast, many adult brood parasites of social insects enter their host colony, where they proceed to lay eggs which are cared for by the host workers. This strategy, termed inquiline or 'social' parasitism, is seen in a number of ants, bees and wasps, and differs from avian brood parasitism in that there is a prolonged period of interaction between the adult parasite and its hosts (Lenoir *et al*. 2001; Cervo 2006).

Unlike adult avian brood parasites, which have very little contact with their hosts, adult social parasites face the challenge of entering a host colony and reproducing, without being killed by the hosts. In many social parasites, this is accomplished via subversion of the hosts' nest-mate recognition system. For instance, parasites may deceive hosts into allowing them access to their colony by mimicking the host (e.g. via the synthesis of host-specific compounds), or else may avoid detection by concealing or diluting recognition markers that could otherwise be used to identify the parasite as an alien, a strategy known as chemical insignificance (Lenoir *et al*. 2001; Lorenzi 2006).

[4] An alternative solution is to translocate hosts to the parasite colony where they can rear parasite offspring, a strategy known as dulosis (or 'slave-making') which occurs in several ant species (D'Ettorre & Heinze 2001).

In a number of social parasites, however, usurpation of the host colony is achieved by aggression, rather than stealth or deception. Aggressive usurpation, whereby parasites engage their hosts in physical combat in an attempt to gain access to the host colony, is observed in several social parasites in the wasp subfamilies Vespinae and Polistinae, as well as in a number of slave-making ants (Reed & Akre 1983; D'Ettorre & Heinze 2001; Cervo 2006). While studies of other host-brood parasite interactions have tended to focus on mechanisms of host detection and parasite crypsis (see above), these aggressive usurpation attempts offer a valuable opportunity to explore information gathering between heterospecifics in a different context, namely assessment during competition between heterospecific rivals. In this thesis, I consider the potential for information gathering during usurpation fights between the social parasite *Polistes semenowi* and its hosts. In the following section, I provide a brief introduction to *P. semenowi*, before exploring the potential role of information gathering in conflict over colony usurpation between the parasite and its hosts.

1.3.2 An introduction to *Polistes semenowi*

Within the genus *Polistes*, there are three obligate socially-parasitic species – *P. semenowi*, *P. sulcifer* and *P. atrimandibularis* - which together form a monophyletic clade (Carpenter 1997). All three species are very sparsely distributed, though may be locally abundant at certain sites within their native range, which includes areas within the Mediterranean and Caspian basins (Cervo 2006). As in other inquiline brood parasites, *Polistes* social parasites live and reproduce within a host colony and rely on the hosts to rear their offspring . However, there are differences between the species in the degree of specialisation in their use of hosts, and in the strategies used to gain access to the host colony (reviewed in Cervo 2006).

Of the three parasite species, *P. semenowi* is the least studied (Cervo 2006). Knowledge of basic aspects of the species' behaviour and life history come from field studies in southern Spain by Almond (2007) and a series of lab studies on populations in central Italy by Zacchi *et al*. (1996) and Lorenzi *et al*. (2004). *P. semenowi* is considered a specialist parasite of *P. dominulus* (Cervo 2006). The parasite invades host colonies in the late pre-emergence phase of the colony cycle when host brood are well-developed,

but before the emergence of the first workers (Figure 1.4a,b). As noted above, *P. semenowi* adopts an aggressive invasion strategy, engaging foundresses sequentially in sustained physical combat until they submit or flee from the nest (Zacchi *et al*. 1996; J. P. Green, pers. obs.). Behavioural observations by Zacchi *et al*. (1996) suggest that aggression by the parasite towards the dominant foundress is particularly severe. However, *P. semenowi* does not usually kill the dominant foundress, something that is commonly observed in colony usurpation by *P. sulcifer* (Zacchi *et al*. 1996; Cervo 2006). In common with *P. sulcifer* and *P. atrimandibularis*, *P. semenowi* is morphologically adapted for fighting (Cervo 2006). The parasite has larger, thicker mandibles and enlarged femora, which assist in physical combat with hosts, as well as a thick cuticle that may protect the parasite against host biting and stinging attempts (Cervo 2006; Figure 1.4c).

Observations in the wild indicate that the usurpation of a nest by the parasite often triggers temporary nest abandonment by the hosts for up to 48 hours (J. P. Green, pers. obs.). In a small number of cases, host abandonment is permanent, and Almond (2007) has suggested that nest abandonment may represent a specific adaptation against parasitism in the host species. In the majority of cases, however, hosts return to the nest, they are seen to respond submissively towards the parasite, which assumes the status of dominant reproductive within the colony (Zacchi *et al*. 1996; J. P. Green, pers. obs.). Research by Lorenzi *et al*. (2004) indicates that the parasite's integration within the host colony may be facilitated by adoption of the colony odour, which the parasite appears to acquire by rubbing itself on the nest surface (Lorenzi 2006).

Following usurpation, the parasite empties cells of immature host brood and replaces them with its own eggs. Developing parasite brood are provisioned by host foundresses and newly-emerged host workers (Cervo 2006). The extent of parasite participation in colony activities is unclear. The parasite rarely leaves the nest and does not forage. On the nest, the parasite actively checks the contents of cells, but does not provision brood or engage in nest building (J. P. Green, unpubl. data). The overt aggression displayed by the parasite during usurpation rapidly subsides as it becomes established within the host colony, and the parasite then becomes less aggressive towards hosts than is a dominant host foundress on an unparasitised nest (Almond 2007). Nonetheless, the parasite does

interact with its hosts, and is frequently observed to mount hosts and subject them to long periods of grooming (J. P. Green, pers. obs.; Figure 1.4d).

In common with other *Polistes* social parasites, newly-eclosed *P. semenowi* offspring do not engage in any cooperative behaviours but instead leave the nest to mate (Cervo 2006). Details of mating behaviour in this species are scarce, but in other *Polistes* social parasites, males and females migrate to high-altitude areas where mating occurs (Cervo & Dani 1996). Females then enter diapause, emerging in the spring approximately one month after the start of host nest founding (Cervo 2006).

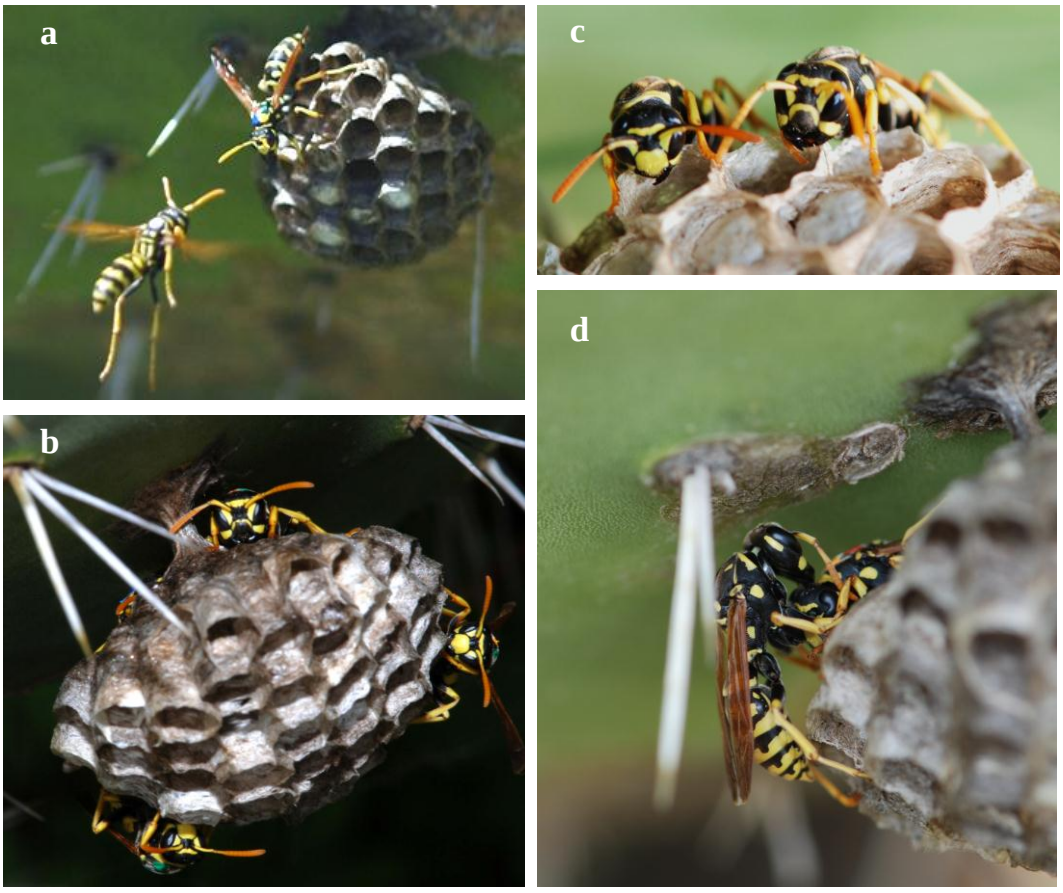


Figure 1.4 (a) *P. semenowi* female approaching a *P. dominulus* nest during the initial usurpation attempt (photograph by Jasper van Heusden). (b) A parasitised *P. dominulus* colony, with *P. semenowi* (top). (c) *P. semenowi* next to a host foundress – note the larger, squarer head of the parasite. (d) The parasite grooms a host foundress.

1.3.3 Part II research aims

Host usurpation by *Polistes* social parasites can be viewed as a contest in which parasite and hosts compete for control of a non-divisible resource (the nest). As in contests between conspecifics, information gathering by heterospecific contestants could be used to reduce the costs associated with escalated fighting. However, while the basic elements of these fights have been described (e.g. Zacchi *et al*. 1996), little is known about the scope for, and importance of, information gathering, including assessment of the agonistic abilities of parasites, hosts, or both. In Part II of this thesis (Chapter 6), I explore the potential for information gathering in *P. semenowi – P. dominulus* usurpation contests. In particular, I investigate whether the parasite clypeal pattern functions as a signal of RHP to hosts, as has been suggested by previous authors (e.g. Ortolani *et al*. 2010). I also investigate the rules of assessment underpinning usurpation contests to establish whether strategic decisions made during contests are based on information about an individual's own agonistic ability and/or the ability of its rival(s).

1.4 Information gathering, conflict and cooperation

The ability of individuals to gather information about aspects of their environment is essential for selecting the optimal behavioural response in situations of conflict, but is also fundamental to the evolution and maintenance of cooperative behaviours (Griffin & West 2003; Kokko 2003; Gardner *et al*. 2010). In the final section of this thesis, I consider the importance of information gathering in the distribution of fitness benefits among members of cooperatively breeding groups. Below, I provide a brief overview of the importance of information gathering in the evolution of cooperation. I then move to outline the role of information in the distribution of direct fitness benefits among co-operators, before concluding this section with an outline of my research aims.

1.4.1 Information and cooperation

The evolution of cooperation is one of the most hotly-debated topics in biology (Gardner *et al*. 2010). West *et al*. (2007) define a cooperative behaviour as one that 'provides a benefit to another individual (recipient), and which is selected for because of

its beneficial effect on the recipient.' Inclusive fitness theory (Hamilton 1964) indicates that natural selection can favour cooperation via effects on direct fitness and/or indirect fitness (Gardner *et al.* 2010). Direct fitness benefits to cooperation may arise from enforced cooperation, whereby co-operators receive rewards and selfish individuals are punished, or else as a by-product or automatic consequence of cooperation (Gardner *et al*. 2010). Central to the stability of mechanisms enforcing cooperation is the ability of individuals to gather information about the behaviour of others (e.g. Zahavi 1995; Riolo *et al*. 2001; Stevens & Hauser 2003; Cant 2011). For example, the potential for reciprocal interactions to stabilise cooperation requires recognition between donors and recipients (Stevens & Hauser 2003), while the effectiveness of threats or sanctions against selfish individuals depends on the enforcer detecting that selfish behaviour is occurring (e.g. Cant 2011). The importance of information gathering is perhaps most vividly underscored in the controversial 'social prestige' hypothesis of Zahavi (1995), which proposes that the propensity to cooperate functions as an strategic signal of an individual's quality, such that more cooperative individuals are more successful in attracting future potential co-operators and/or mates.

Information gathering is also important in the evolution of cooperation through indirect fitness benefits (Le Galliard & Ferrière 2008). Indirect (or kin-selected) benefits arise when cooperative behaviours enhance the reproductive success of an individual's genetic relatives (Hamilton 1964). Importantly, such indirect benefits can favour the evolution of cooperative behaviours in the absence of direct fitness benefits, providing that the benefit to relatives outweighs the cost of cooperation, according to Hamilton's rule (Hamilton 1964; Gardner *et al*. 2010). Costly cooperative behaviours (i.e. altruistic behaviours) can therefore evolve, but only when the benefits are preferentially directed towards relatives. In some cases, altruism towards relatives may be facilitated by the kin structure of populations, for example where limited dispersal results in stable family groups. In such cases, indiscriminate altruism may be possible, as the majority of recipients will tend to be relatives (Gardner *et al*. 2010, and references therein). However, in other cases, altruism requires that individuals are able to detect and discriminate in favour of relatives. Genetic relatedness is often correlated with kinship (Gardner *et al*. 2010), and a wealth of theoretical and empirical research has focused on the ability of individuals to recognise kin and to discriminate between kin and non-kin

(reviewed in Griffin & West 2003; Gamboa 2004; Gardner *et al*. 2010). Based on these studies, there is good evidence that individuals in a range of social taxa are able to discriminate between group members and non-group members using a variety of phenotypic and environmental cues, and appear to use group membership as a proxy for kinship (Le Galliard & Ferrière 2008). However, the ability of individuals to gather precise information about relatedness based on genetic cues is much more limited (Le Galliard & Ferrière 2008; Gardner *et al*. 2010), which perhaps reflects the potential for greater instability in genetic recognition markers (Gardner & West 2007).

The ability of an individual to gather information about the environment, in particular about its social partners, thus plays an important role in the evolution of cooperation. In situations where the expression of cooperative behaviours is flexible, the information that an individual acquires can help it to make optimal decisions as to what extent and with whom it should cooperate. An important body of theory in this regard is reproductive skew theory, which explores the conditions under which cooperation is favoured, as well as the factors influencing how the benefits of cooperation are distributed among co-operators (Keller & Reeve 1994; Reeve & Keller 2001). In the final section of the thesis, I explore the mechanisms underpinning cooperation in *Polistes dominulus* wasps by testing the predictions from competing models of reproductive skew. Below, I provide a brief overview of reproductive skew models, as well as the difficulties encountered in testing them in *P. dominulus*. A more in-depth introduction to these areas can be found in Chapter 7.

1.4.2 Reproductive skew and information gathering

Within cooperatively breeding groups, reproductive skew refers to the distribution of reproductive benefits among group members, which can vary from an equal distribution (low skew) to a monopoly on reproduction by a single individual (high skew). Reproductive skew models aim to explain this variation in terms of negotiations and/or competition between group members (Reeve & Keller 2001). Traditionally, two main types of skew model have been distinguished: transactional models and tug-of-war (or incomplete control) models. Transactional models assume that skew is the result of a negotiation between the subordinate and its dominant over the size of the subordinate's

reproductive share (Reeve & Ratnieks 1993). In contrast, tug-of-war (or compromise) models assume that an individual's share of reproduction is determined by its competitive ability, with group members competing for reproductive benefits (Reeve *et al*. 1998). An important assumption of reproductive skew models is that individuals are able to detect and respond to social conditions within the group, as well as the conditions faced by independent breeders (Nonacs 2006; Field & Cant 2009). This is particularly important in transactional models, where the size of a subordinate's reproductive share is argued to reflect its scope for indirect benefits and independent breeding (Johnstone 2000). Moreover, Kokko (2003) has argued that the stability of transactions over reproduction requires that individuals are able to gather information about the actions of partners over a behavioural time scale, which is considered necessary to prevent exploitation of negotiations by dishonest individuals (Kokko 2003). Information use is therefore important to the stability of skew models, and limitations on information gathering may have important consequences for tests of reproductive skew theory in cooperative groups, a problem I address briefly below.

1.4.3 Exploring reproductive skew in *Polistes dominulus*

Polistes wasps have provided an important testing ground for skew models (e.g. Field *et al*. 1998; Seppä *et al.* 20002; Liebert & Starks 2006). In the case of *P. dominulus*, previous tests of reproductive skew have concluded that skew is unlikely to be the result of a transaction in which the dominant cedes reproduction to the subordinate, based on the observation that a subordinate's share of reproduction does not vary with its relatedness to the dominant (Queller *et al*. 2000; Liebert & Starks 2006; Nonacs *et al*. 2006). Among the social Hymenoptera, *P. dominulus* is exceptional for the large numbers of unrelated individuals in cooperatively-breeding groups (Queller *et al*. 2000; Zanette & Field 2008). According to transactional skew theory, unrelated individuals occupying a subordinate role should receive more direct reproduction as there is no opportunity for indirect fitness benefits through helping to rear the dominant's offspring. However, it is unclear whether *P. dominulus* is able to discriminate between nest-mates on the basis of genetic relatedness (see above), and a number of studies have suggested that individuals instead discriminate kinship based on whether conspecifics shared the same natal nest in the previous year and on the mean relatedness to natal

nest-mates (reviewed in Gamboa 2004). Crucially, if individuals are unable to distinguish between nest-mates based on relatedness, the decision of previous studies (Queller *et al*. 2000; Liebert & Starks 2006; Nonacs *et al*. 2006) to reject transactional models of skew, based on the finding that skew does not vary with relatedness, may be flawed. Rather, we require a means of generating variation in kinship that is readily detected by nest-mates, and which may be used to negotiate reproductive shares in the event that transactions over reproduction occur.

1.4.4 Part III research aims

In Part III of this thesis (Chapter 7), I test competing models of reproductive skew in *P. dominulus* using social parasitism as a tool to generate variation in relatedness between nest-mates. By comparing skew between unparasitised colonies and colonies parasitised by the social parasite *P. semenowi*, in which average dominant-subordinate relatedness is lower, and dominant-subordinate RHP asymmetries are greater, it will be possible to gain insights into the relative importance of kinship and competition in determining reproduction by subordinates in this species.

1.5 Structure of thesis

In **Chapter 2**, I introduce my study populations and outline general methods that will be used in the subsequent chapters.

In **Chapter 3**, I test for status signalling in a Spanish population of *P. dominulus*.

In **Chapter 4**, I explore the relationship between clypeal pattern brokenness and quality in a natural population of *P. dominulus*.

In **Chapter 5**, I explore factors affecting the development of the clypeal pattern in *P. dominulus*.

In **Chapter 6**, I test for rival assessment in usurpation contests between *P. dominulus* and the social parasite *P. semenowi*.

In **Chapter 7**, I test competing models of reproductive skew in *P. dominulus* using social parasitism as a novel tool to generate variation in relatedness between nest-mates.

In **Chapter 8**, I summarise my main findings and suggest future areas of research.

Chapter 2: General Methods

In this chapter I provide general information about the wasp populations studied in the following chapters. I also provide information about the following methodologies:

- Molecular techniques (Chapters 4 and 7)
- Image analysis (Chapters 4 and 5)
- Clypeal pattern manipulations (Chapters 3 and 6)
- Statistical methods

A variety of other methods are employed in the following chapters. Details of these methods are provided in the relevant chapter.

2.1 Study populations

The data that I present in the following chapters come from studies of *P. dominulus* and *P. semenowi* populations in south-west Spain. I studied both species at rural sites near the coastal towns of Conil de la Frontera, El Almarchal and La Zarzuela in Cádiz Province, Andalucía (Figure 2.1a). These sites consist of areas of arable and pastoral farmland and support high densities of *P. dominulus*, with wasps chiefly nesting on hedges of prickly pear cactus (*Opuntia* sp.) (Figure 2.1b-d).

2.1.1 *P. dominulus*

Due to the warm climate in southwest Spain, the timing of events in the colony cycle is slightly advanced in the Spanish populations compared with that of populations in other, more northerly habitats (described in Reeve 1991). Gynes typically emerge from diapause in February and nest founding begins in late February – early March, depending on weather conditions. Nests are founded by a single foundress, or, more commonly, by a group of co-foundresses. The nest-founding phase, which lasts approximately one month, is characterised by frequent changes in group membership

and nest ownership (Zanette & Field 2011), as well as by intense aggression between foundresses (J. P. Green, pers. obs.). Following nest founding, colonies enter the pre-worker emergence phase of the colony cycle, characterised by increased stability of co-foundress groups. The first offspring eclose in late April – early May (later in cases of prolonged cold or wet weather) and offspring continue to emerge until mid-July. Data on the fate of offspring eclosing at different times in the season indicate that females eclosing up to mid-June are most likely to remain on the nest as workers, whereas those eclosing after mid-June are most likely to mate and overwinter, founding nests in the following spring (Leadbeater *et al*. 2011). After the last offspring emerge in July, colonies dissolve and reproductive females and males leave to mate. The timing of mating and female entrance into diapause has not been investigated in the Spanish population but is probably broadly similar to that in other populations (i.e. mid- to late Autumn).

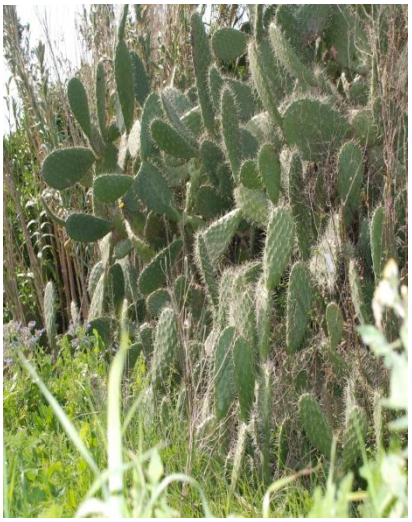
During the nesting phase, *P. dominulus* nests suffer moderate rates of predation by rats, birds and ants. Colonies are also vulnerable to attack by parasitoids, with the parasitoid wasp *Latibulus argiolus* (Rossi 1790) in particular reaching high densities at several sites (Z. Ács, pers. comm.). Finally, as noted below, the Spanish populations of *P. dominulus* are also subject to attack by the social parasite *Polistes semenowi*. Neither of the other two species of social parasite (*P. sulcifer* or *P. atrimandibularis*) have been found at the Spanish sites, however.

2.1.2 *P. semenowi*

Little is known in any detail about the movements and behaviour of *P. semenowi* at the Spanish sites. Mated females are first observed at the sites in mid-April during the pre-worker emergence phase of the host (*P. dominulus*) colony cycle. During the years in which the following studies were undertaken (2008-2010), the density of parasites at each site was low. Consequently, host usurpation attempts were observed only rarely. As a result, there is no information on the number of usurpation attempts individual parasites make, and no record of attempts that have failed. Therefore, parasites are often identified for the first time following a successful usurpation attempt.

a

c



b

d

Figure 2.1 Illustrations of field sites in Spain where *P. dominulus* and *P. semenowi* were studied. (a) Populations were studied at rural sites in Cádiz province, Andalucía (arrow indicates general area of study). (b) Hedges of prickly pear cactus (*Opuntia* sp.) at one of the sites. (c) Close-up photograph of a prickly pear hedge, where *P. dominulus* is found to nest at high densities. (d) *P. dominulus* female building her nest on the cactus during the nest founding phase of the colony cycle.

The majority of usurpation attempts occur in late April – early May prior to the emergence of the first offspring on host nests; a small number of parasites are found on host nests in mid-April but tenure on these nests is usually short (J. P. Green, pers. obs.). Previous observations have indicated that host nests successfully usurped by parasites tend to be larger and more developed than those remaining unparasitised (Shreeves *et al*. 2003), though it is unclear whether this reflects active selection of larger nests by parasites. Once established on nests, *P. semenowi* assumes the role of dominant reproductive, with the first parasite offspring eclosing approximately 35-40 days after

nest usurpation (J. P. Green, pers. obs.). Parasitised colonies are vulnerable to attack by predators and parasitoids (J. P. Green, pers. obs.) and survival during the season does not differ from unparasitised nests (Shreeves *et al*. 2003). The fate of adult parasites and their offspring at the end of the season has not been investigated in the Spanish populations. In other *Polistes* social parasites, males and females ascend to high-altitude areas to mate, after which females enter diapause (see Chapter 1) and *P. semenowi* offspring likely display similar behaviours.

2.2 Molecular techniques

In Chapters 4 and 7, I estimated reproductive success for *P. dominulus* and *P. semenowi* using microsatellite analyses. Adults (foundresses and parasites) and brood of both species were genotyped at 9 microsatellite loci using the following set of fluorescently-labelled primers: Pbe128TAG, Pdom1jc, Pdom2jc, Pdom25jc, Pdom7, Pdom20, Pdom122, Pdom127b and Pdom140 (for details of microsatellites and primers see Appendix A). All the primers were initially developed for use in *P. dominulus* (Henshaw 2000; Leadbeater *et al*. 2010, 2011), with the exception of Pbe128TAG, which was developed in *P. bellicosus* (Strassmann *et al*. 1997). *P. dominulus* and *P. semenowi* are closely related (Carpenter 1997) and results from initial genotyping runs, as well as from earlier work by Almond (2007), have shown that all nine primers also amplify successfully in *P. semenowi*.

2.2.1 DNA extraction

DNA from adult wasps was extracted from a whole leg (or tarsal sample, where available). Depending on the developmental stage of brood, DNA was extracted from a whole egg or the head capsule of a pupa. To extract DNA, whole tissue samples were bathed in 50µl of buffer solution containing 10mM Tris-Cl (pH 8.2), 1mM EDTA, 25mM NaCl, and 200µg/ml Proteinase K (adapted by J. M. Carruthers from Gloor *et al*. 1993). Samples were then incubated at 57^{o}C for 40 minutes, then at 95^{o}C for two minutes to inactivate the Proteinase K. Results of initial runs indicated that a DNA purification step was unnecessary.

2.2.2 PCR

Multiplex polymerase chain reactions were performed on a Peltier Thermal Cycler, using nine fluorescently labelled primers. Each reaction contained 1µl of the template DNA solution, which was evaporated before adding a 2µl mix of the nine primers, and 2µl of PEQ Gold Hot Start Mix Y (dNTP, Taq DNA polymerase, NH_4^+ buffer and $MgCl_2$ mix). A drop of mineral oil was added to each well to prevent evaporation of the reaction mixture during PCR. Each plate contained a positive and negative control in order to test for consistency in primer amplification. Plates were transferred to the thermal cycler, and the following programme run:

94°C	3 minutes	
94°C	30 seconds	x 35 cycles
57°C*	30 seconds	
72°C	30 seconds	
72°C	10 minutes	

*Annealing temperature of the primers

2.2.3 Sizing and scoring of PCR products

PCR products were separated by size using an Applied Biosystems 3730 automated capillary sequencer. For each sample, 1µl PCR product was added to a 10µl mix of formamide and size standard (Applied Biosystems GeneScan LIZ 500). Samples were incubated at 95^{o}C for three minutes to separate the DNA strands. Following incubation, samples were placed immediately in ice for five minutes. Samples were then sequenced at the NERC Biomolecular Analysis Facility at the University of Sheffield, UK. Products were visualised using the software GeneMapper 3.7 (Applied Biosystems). The nine loci used could be distinguished based on size range or dye colour. When viewed in GeneMapper, each locus produced a distinct band of size peaks. As alleles at all loci were separated by multiples of three base pairs, allele peaks were scored by rounding the absolute peak score to the nearest expected allele value. In cases where alleles were intermediate between two expected values, samples were re-run and re-

scored. Maternity assignment and estimation of relatedness using the microsatellite loci are discussed in the relevant chapters.

2.3 Image analysis

In Chapters 4 and 5, I calculated brokenness scores for *P. dominulus* clypeal patterns using the image analysis software MATLAB (version 7.1, The MathWorks Ltd., Cambridge, UK). I began by converting digital images of the clypeus into a standard 60 x 30 pixel bitmap. Following Tibbetts (2010), I then calculated brokenness as the standard deviation of the number of black pixels in each vertical column of the bitmap from pixels 6 to 55 along the horizontal gradient of the clypeus (Figure 2.2). Pixels 1-5 and 56-60 were excluded as these contained black pixels corresponding to the border of the clypeus, rather than to the patterns on the clypeus.

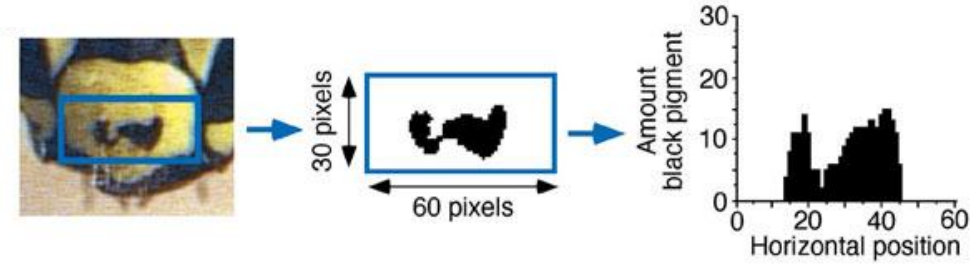


Figure 2.2 Quantifying clypeal pattern brokenness. A rectangular section of the clypeus containing the clypeal pattern is cropped from the original photograph and converted into a 60 x 30 pixel bitmap. The number of black pixels in each column is counted from columns 6-55. Brokenness is then calculated as the standard deviation of these counts. Figure adapted from Tibbetts & Dale (2004).

2.4 Clypeal pattern manipulations

In Chapters 3 and 6 I explored the signal function of clypeal patterns in *P. dominulus* and *P. semenowi* by testing receivers responses to experimental manipulation of the patterns, performed with Humbrol enamel paint. A potential concern with performing

manipulations of this kind is that any response by the receiver may be the result of a reaction to the presence of paint, rather than to a perceived change in signal intensity. *Polistes* wasps are thought to make use of both olfactory and visual cues during social interactions (see Chapter 1), and distortions of these cues by specific properties of the paints used could provoke abnormal responses that may mask responses to changes in the signal. The experiments I present in Chapters 3 and 6 are designed to control for general effects of paint on normal behavioural cues. In Chapter 3, receivers are given the choice of interacting with one of two signallers, both of which have experimentally-altered clypeal patterns. In Chapter 6, a treatment group in which clypeal patterns are concealed with paint is complemented with a control group in which a similar area of yellow cuticle elsewhere on the head is concealed. By using these designs, I was therefore able to control for receiver responses to the presence of paint while testing for responses to the specific manipulations.

However, in order to minimise any responses elicited by the paint, I also attempted to match the spectral reflectance of the paints to that of the wasp cuticle. To do this, I compared the spectral reflectance curves of black and yellow paints with reflectance curves of *P. dominulus* cuticle (averaged over 5 individuals) using a USB2000 spectrometer (Ocean Optics, BD, Duiven, The Netherlands). Comparisons of the spectral reflectance data for black paint and black cuticle showed no difference in the shape of the reflectance curves for the paint and the cuticle over a range of 400-700nm. Of the two yellow paints examined ('Yellow' and 'Zinc Chromate'), the curve for Zinc Chromate was most similar to that of the yellow cuticle. However, whereas cuticular reflectance increased gradually from 400-700nm, the reflectance of the paint increased sharply between 500-525nm, resulting in a more pronounced 'S-shaped' curve (Figure 2.3). To determine whether wasps could discriminate between the yellow paint and cuticle, I used the reflectance data to calculate the distance in colour space between the paint and the cuticle using the bee colour hexagon (Chittka 1992). Honeybee spectral sensitivities were used in the calculations as these are very similar to the sensitivities of *P. dominulus* receptors (Peitsch *et al*. 1992). The distance between the paint and cuticle was calculated as 0.03, indicating that discrimination between the paint and cuticle by wasps in the pattern manipulation experiments is unlikely (Théry & Casas 2002).

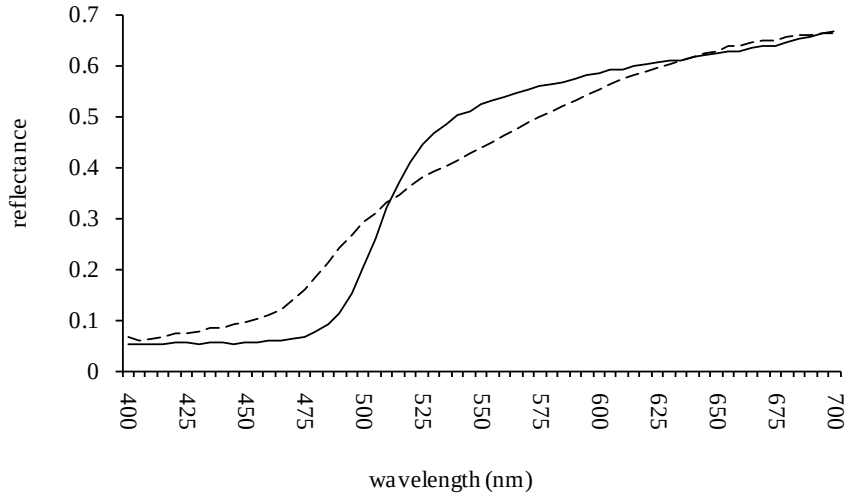


Figure 2.3 Reflectance curves for *P. dominulus* yellow cuticle on the clypeus (dashed line) and the yellow paint ('Zinc Chromate') used in the manipulations (solid line). Curves are calculated relative to a barium sulphate standard. Curves are of broadly similar shape, with an increase in reflectance from 400nm to 700nm. Note, however, the steeper increase in paint reflectance from 500-525nm compared with the cuticle.

2.5 Statistical methods

All statistical analyses in Chapters 3 – 7 were performed using the statistical software R version 2.9.2 (R Core Development Team, 2009). Throughout, I make use of a variety of linear and generalised linear modelling techniques. I therefore provide a short introduction to these techniques below. Where other techniques are used, these are described in the Statistical Analysis section of the relevant chapter. For parametric tests, I ensured that assumptions about the distribution and variance of the response data were met before using the tests (Zuur *et al*. 2009a). Anderson-Darling tests were used to test for normality of residuals, and Levene's tests were used to test for constancy of variance. In cases where these assumptions were not met for continuous response variables, transformation of the data was first attempted. Where transformation of the data was unsuccessful or else was not attempted (e.g. in the case of count or proportion data), nonparametric tests were used. For all analyses, unless otherwise stated, $\alpha = 0.05$. Arithmetic means are quoted throughout ± 1 standard error.

2.5.1 Linear modelling techniques

In the following chapters, I make use of a number of linear modelling techniques. An introduction to these techniques can be found in Crawley (2007), Bolker *et al*. (2008) and Zuur *et al*. (2009b). For each analysis, the appropriate model was determined by the nature of the response variable. For continuous response variables, linear models (LM) were used. LM encompass parametric techniques including Normal regression, ANOVA and ANCOVA and are suitable for analysis of continuous response variables with normally-distributed residuals and constant variance. In cases where the response variable was binary, or in the form of counts or proportions, generalised linear models (GLM) were used. GLM are a family of non-parametric analyses, for which particular error structures and link functions may be specified depending on the form of the response variable. Where the response variable was in the form of counts, GLM with a Poisson error structure and a log link function were used. (For highly aggregated counts, a negative binomial error structure was used where model comparisons based on log-likelihood tests indicated that this provided a better fit to the data than Poisson errors.) Alternatively, where the response variable was binary, or in the form of proportions, GLM with a binomial error structure and a logit link function were used.

A critical assumption of many statistical tests is that data points are independent (i.e. there is no pseudoreplication in the data; Zuur *et al*. 2009a). For several analyses in the following chapters, however, the data are not independent (e.g. individuals sampled from the same nest, or winners and loser sampled from the same contest). In these analyses, I therefore used linear mixed-effects models, in which the source of pseudoreplication (e.g. nest, contest) can be fitted as a random effect, and the amount of variance in the data due to differences between the levels of random effects can be quantified (Zuur *et al*. 2009b). In cases where the response variable had normal residuals and constant variance but exhibited pseudoreplication, linear mixed effects models (LMM) were used. Alternatively, where the response variable was binary, or in the form of counts or proportions, generalised linear mixed effects models (GLMM) were used, together with the appropriate error structure and link function (Bolker *et al*. 2008). In some analyses using GLM and GLMM, a comparison of the residual deviance and residual degrees of freedom in the model output pointed to overdispersion in the

data (i.e. unexplained variation beyond the variance assumed by the specified error structure). In such cases, models were re-run, this time specifying a quasi-binomial or quasi-Poisson error structure, following Crawley (2007).

In the above models, it is possible to fit both continuous and categorical explanatory variables. It is also possible to fit interactions between two or more variables, where it is suspected that the effect of one variable is dependent on the level of a second variable. To analyse data using linear models, I began by fitting all relevant explanatory variables (including random effects where appropriate) and interactions in a maximal model. Model simplification then proceeded by backwards deletion of nonsignificant terms until further removals led to a significant ($p < 0.05$) increase in deviance. This was assessed by comparing the model with and without the term in question, using (1) tabulated F values for LM and GLM with quasi-Poisson or quasibinomial errors; (2) χ^2 values for GLM and GLMM with Poisson or binomial errors, and (3) Log-likelihood tests for LMM (Crawley 2007). In all cases, significance levels are reported for the addition of nonsignificant terms, and removal of significant terms from the minimum adequate model. In all analyses, models were checked for goodness of fit to the data using procedures outlined in Crawley (2007). Both the maximal and minimum adequate models were checked for constancy of variance and normality of residuals (where appropriate) and for the presence of influential outliers (Crawley 2007).

2.5.2 Statistical power

The statistical methods employed in this thesis are based on null-hypothesis significance testing. Statistical power refers to the probability that a null-hypothesis significance test will reject the null hypothesis when it is false. The statistical power of a test depends on both the size of the effect under investigation and the sample size: all else being equal, power will be low where the effect size is small and/or the sample size is small. In the following data chapters, small putative effect sizes and small sample sizes act to reduce the power of a number of statistical tests. In particular, in several cases, low power may account for the failure of analyses to detect statistically-significant effects. In these cases, the issue of statistical power is considered explicitly in the Discussion sections of the relevant chapters.

Chapter 3: Status signalling in a Spanish population of *Polistes dominulus*

The material in this chapter is published:

Green, J. P. and Field, J. 2011. Inter-population variation in status signalling in the paper wasp *Polistes dominulus*. *Animal Behaviour*, **81**, 205-209.

3.1 Introduction

Contests between individuals over resources can inflict significant costs on both contestants, in terms of time, energy and the risk of injury or death. Theory suggests that the costs of escalated conflicts will be minimised if individuals can assess the competitive ability of their opponents at the start of contests, and thereby predict the likely outcome of a fight (Maynard Smith & Harper 1988). Status signals have been argued to play a role in resolving contests over relatively low-value resources, where the fitness pay-offs of acquiring the resource are less than the fitness costs of escalated fighting (Rohwer 1975; Maynard Smith & Harper 1988). Visual status signals are small patches of colour that convey information about an individual's competitive ability, or resource holding potential (RHP), to a rival. These signals are 'conventional' in the sense that they are not causally linked to RHP, and are furthermore considered to be relatively cost-free to produce, in contrast with indices and strategic signals (Maynard Smith & Harper 2003; Chapter 1, section 1.2.2).

Empirical support for status signalling has come largely from studies of passerines (Senar 2006) and lizards (Whiting *et al*. 2003). Recently, however, a number of studies have suggested that status signalling also operates in the paper wasp *Polistes dominulus* (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). In North American populations, the amount of disruption (brokenness) in black patterns on the clypeus is argued to signal RHP in during competitive interactions (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010), with social costs enforcing honesty (Tibbetts & Dale 2004; Tibbetts & Izzo 2010). However, recent work has indicated that receiver responses to the signal depend not only on the level of RHP that

is signalled but also on the RHP of the receiver (Tibbetts *et al*. 2010). For example, in contests over food, wasps with more broken patterns do not avoid rivals with less broken patterns, consistent with a process of mutual assessment (Tibbetts *et al*. 2010).

However, while work on *P. dominulus* in North America has found consistent support for the use of status signals, two studies in the species' native Eurasian range have failed to find evidence for a link between clypeal patterning and RHP. In Italy, *Cervo et al*. (2008) found that hierarchical rank in foundress groups was not correlated with brokenness of the clypeal pattern. In a second population in Spain, Zanette & Field (2009) found only a weak correlation between hierarchical rank and the size of the clypeal pattern. These findings appear to cast doubt on the function of clypeal patterns as signals of status in Europe and indicate that there may be geographic variation in status signalling in *P. dominulus*. However, support for this hypothesis requires a manipulative test of status signal use in Europe, which to date has been lacking.

Here, I test for status signalling in the Spanish population of *P. dominulus* studied by Zanette & Field (2009). In testing for a signal function in the clypeal pattern, I follow the experimental procedure of Tibbetts & Lindsay (2008). The demonstration of status signalling requires that manipulation of the putative signal results in a change of behaviour in the receiver, consistent with the idea that the signal conveys information about individual RHP. This is not straightforward, for two reasons (Senar 1999). Firstly, a change in receiver behaviour may be due to difficulties with individual recognition following manipulation of the signaller (Shields 1977). Secondly, studies using familiar individuals run the risk of confounding the demonstration of status signalling with the demonstration of anti-cheating mechanisms, which may interfere with receiver responses if the signaller's true status is known to the receiver (Senar 1999). It is important therefore that the receiver has not interacted with the signaller prior to the manipulation, and that the receiver possesses no information about the signaller's RHP, other than what is signalled. The design used here and by Tibbetts & Lindsay (2008) fulfils these requirements: focal wasps were presented with a choice of two food sources, each guarded by a potential rival with artificially enhanced or reduced clypeal patterns. These 'guards' had not previously interacted with the focal wasp. Furthermore, the guards were presented dead to prevent focal wasps detecting any incongruence

between behaviour and advertised status. If wasps do use clypeal patterns to assess rivals in a contest situation, focal wasps are expected to avoid the high-status guard, and instead challenge the low-status guard, as seen in Tibbetts & Lindsay (2008).

3.2 Materials and Methods

3.2.1 Focal wasp and guard collection

I collected all wasps in early May 2010 at sites around Conil de la Frontera (Cádiz Province, Spain). Both focal wasps and guards were foundresses collected from different nests in the pre-emergence phase of the colony cycle. I transferred all wasps to large plastic containers (25 x 16 x 16cm) where they were housed for 48 hours, during which time they were provided with *ad libitum* water and sugar cubes. In each trial, I selected focal wasps and guards that had been housed in separate containers and collected from sites at least 3km apart in order to minimise the likelihood of previous interaction. All focal wasps used in trials had 0 clypeal spots, while guards had a variable number of clypeal spots (0-2). Twenty-four hours prior to the choice trial, I placed focal wasps in individual plastic tubes without food. A period of starvation was enforced to ensure that focal wasps were sufficiently motivated to approach the food source during the trials.

3.2.2 Pre-trial guard manipulation

Guards were killed by freezing. I paired guards by the number of clypeal spots (0,1 or 2) and by wing length (measured using digital callipers). I then used Humbrol enamel paint to experimentally alter the clypeal patterns of the guards. The active signal component of the clypeal pattern is the amount of disruption, or 'brokenness'. However, when experimentally manipulating patterns, previous studies have altered the number of clypeal spots, as spot number is argued to be a good proxy for brokenness (Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). By altering the number of clypeal spots, I manipulated one guard of each pair to signal a higher status while the other guard was manipulated to signal a lower status (Figure 3.1). In all, three combinations of guard clypeal patterns were used in the choice trials. Wasps with 0 spots were painted to

produce guards with 1 and 2 spots respectively. Wasps with 1 spot were painted to produce guards with 0 and 2 spots, and wasps with 2 spots were painted to produce guards with 0 and 1 spot. A total of 13 guard pairs were used in the trials.

3.2.3 Choice trials

I carried out choice trials in a rectangular arena (6cm wide x 7cm long) with a convex semicircle at one end. At the other end, a sugar cube with a freeze-killed guard on top was placed in each corner (Figure 3.2a). Immediately prior to the trial, I placed the focal wasps inside a covered tube at the semicircular end of the arena for 5 minutes in order to acclimatise it to the arena. I then released the focal wasp into the arena and recorded its choice of sugar cube. I considered the focal wasp to have made a choice when it approached a sugar cube and began to eat (Figure 3.2b). Wasps were given 30 minutes to make a choice; the median time from release until eating was 50s (range: 2s – 28 minutes). Between trials, I replaced both sugar cubes in order to remove any cues left by previous wasps, which may have influenced the focal wasp's choice. In total, 149 trials were run, with each focal wasp tested only once during the course of the experiment.

3.2.4 Statistical analyses

I tested whether wasps preferred to challenge the high-status or low-status guards using Pearson's χ^2 test. To test the effect of specific pairings of guard clypeal patterns (i.e. 0 vs. 1, 0 vs. 2 and 1 vs. 2) on wasps' choices I used a GLMM with binomial errors. Wasp choice was the binary response variable (0 = low-status guard; 1 = high-status guard) and guard pattern pair was the explanatory variable. Although guards were matched for size, I included the size difference between the guards as a second explanatory variable to test whether small differences in size influenced guard choice. Whether the chosen guard was in the left or right corner of the arena was also included as a third explanatory variable. Because the same guard pairs were used across multiple trials, it was necessary to control for any potential responses of the focal wasp to specific guard pairings, as any such reactions may exaggerate or obscure general responses to different guard pattern pairings. To control for potential effects of specific guard pairings, I therefore fitted individual guard pair as a random effect in the model.

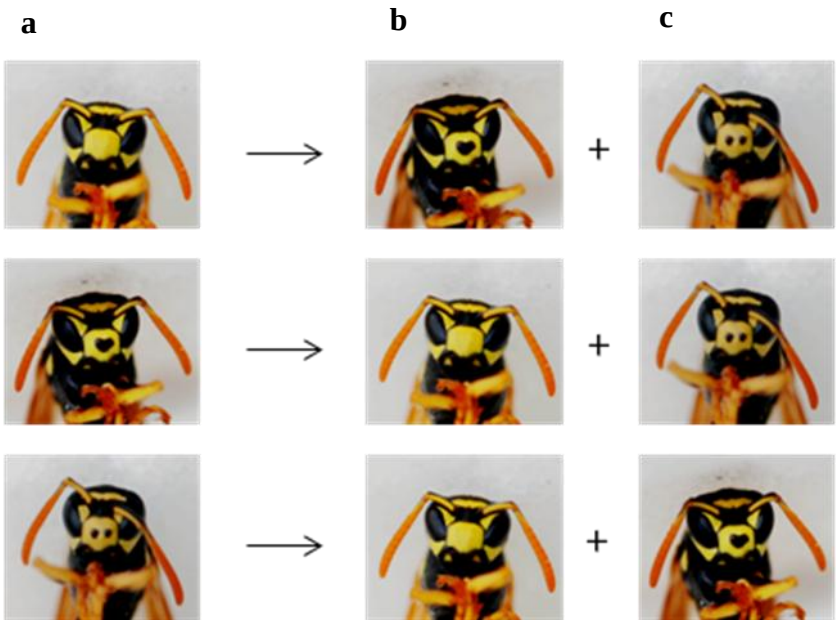


Figure 3.1 Guard clypeal pattern manipulations. Guards were paired by original number of clypeal spots (a). For each pair, paint was added to the clypeus to make a 'low-status' guard (b) and a 'high-status' guard (c).

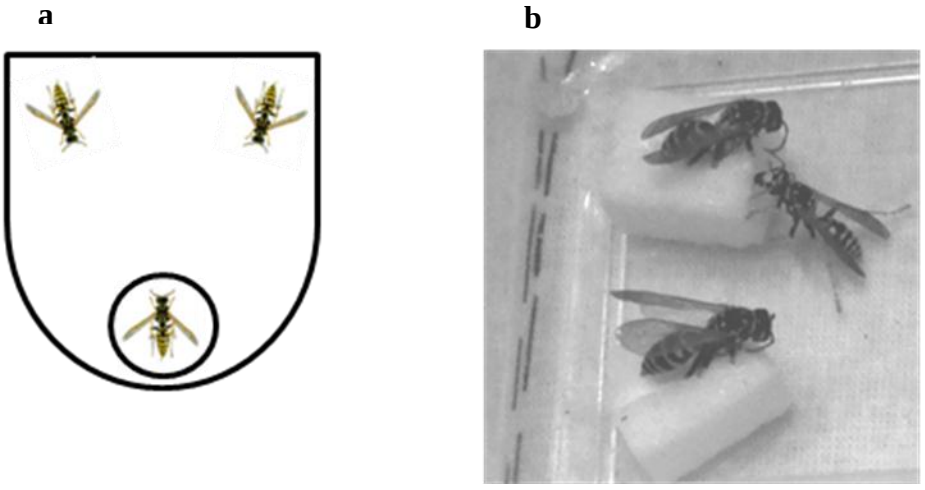


Figure 3.2 Choice trial set-up. The focal wasp was released into the arena after 5 minutes within a covered tube and allowed to choose between two sugar cubes, each with a guard on top (a). A choice was considered to have been made when the focal wasp ate at one of the sugar cubes (b).

3.3 Results

In 86/149 trials, the focal wasp was observed to make a choice within 30 minutes. Results of the analysis showed that focal wasps did not challenge the low-status guard significantly more often than the high-status guard (Pearson's χ^2_2 = 1.71, p = 0.43; Table 3.1). The same result was obtained when considering only trials in which focal wasps chose between 0 vs. 1 and 0 vs. 2 spot guards (Pearson's χ^2_2 with Yate's correction = 0.01, p = 0.94). This indicates that the lack of preference for the low-status guard is not the result of a specific failure to choose between 1 vs. 2 spot guards, which might be expected if mutual assessment is occurring (i.e. 0-spot focal wasps might not be motivated to choose between 1 and 2 spot guards, if both represent superior competitors). Rather, this result demonstrates a general failure to choose between guard clypeal patterns, irrespective of the specific combination of guards.

Analysis of factors influencing the focal wasp's choice of the low-status or high-status guard showed no influence of guard position in the left or right side of the arena on focal wasp choice (χ^2_1 = 1.15, p = 0.28). However, the analysis revealed a significant interaction between the guard pattern pairing and guard size on the choice made by focal wasps (χ^2_2 = 31.01, p < 0.001). When presented with guard pairs with either 0 vs. 1 spot or 0 vs. 2 spots, wasps choosing the smaller guard were more likely to choose the guard with more clypeal spots. However, this tendency is not observed among wasps choosing between guard pairs with 1 or 2 clypeal spots. To analyse the effects of guard clypeal pattern and size on focal wasp choice in more detail, I re-ran the model without the interaction. In the absence of the interaction, neither guard pattern pairing nor size had a significant effect on focal wasp choice (clypeal pattern pair: χ^2_2 = 1.52, p = 0.47; size: χ^2_1 = 0.08, p = 0.77). Thus, when all guard pair types are considered, focal wasps showed no preference for either the smaller or larger guard. Similarly, when guard size is not considered, the focal wasps' choice of the low-status or high-status guard did not depend on the specific combination of clypeal patterns presented by the guard pair.

During the trials, 63 focal wasps did not eat at either sugar cube. To test whether the likelihood of eating (i.e. the likelihood of approaching either guard) was influenced by the particular combination of guard patterns, I used a second GLMM where decision to

eat was fitted as the binary response variable. Guard clypeal pattern pairing and guard size (taken as the size of the smallest guard in a pair) were fitted as explanatory variables and individual guard pair was again added as a random effect. The analysis revealed no significant effect of either guard clypeal pattern pairing or guard size on the decision to eat (guard clypeal pattern: $\chi^2_2 = 0.69$, $p = 0.41$; guard size: $\chi^2_1 = 0.05$, $p = 0.83$). Furthermore, the interaction between guard clypeal pattern and size did not influence the probability of eating ($\chi^2_2 = 0.34$, $p = 0.57$). Therefore, the decision to approach either guard and eat the sugar was not influenced by the relative size of the guards or by the particular combinations of guard patterns (i.e. wasps were equally likely to eat when presented with 0 vs. 1, 0 vs. 2 or 1 vs. 2-spot guards).

These results show that focal wasps do not prefer to challenge the low-status guard. To determine whether this was a result of the experimental set-up used, I carried out another set of trials in which wasps were given the choice between a sugar cube guarded by a 0-spot wasp and an unguarded cube. If wasps can perceive the presence of a guard, then they should prefer to feed at the unguarded sugar cube in order to avoid competition. I found that wasps were significantly more likely to feed at the unguarded sugar cube (binomial test, $n = 35$, 69%, $p = 0.04$). This result is important as it demonstrates that wasps perceive the presence of another wasp in the set-up, and so could use information about the guard's phenotype to choose between guards.

Table 3.1 Numbers of focal wasps challenging high-status and low-status guards

Guard spots	Low-status guard	High-status guard
0 vs. 1	4	4
0 vs. 2	18	14
1 vs. 2	19	27
Total	41	45

3.4 Discussion

I found no evidence that wasps assess conspecifics via visual status signals in a foraging context. When given the choice of two food sources, one guarded by a high-status guard and the other by a low-status guard, focal wasps did not show a preference for feeding near the low-status guard. This suggests that the decision to challenge a particular rival is made without reference to the rival's clypeal patterns, in particular the number of clypeal spots. The analysis revealed a significant interaction between guard size and the guard clypeal pattern pairing, though this is difficult to interpret in light of previous work. The finding that individuals choosing high-status guards from certain pairings (0 vs. 1 spot or 0 vs. 2 spots) tended also to choose the smaller guard could be seen as evidence for rival assessment, with individuals attempting to minimise competition by choosing opponents of smaller size when clypeal patterns indicate high status. However, it is not clear why these effects are seen only in these pairs of guard patterns, and not in the choice trials involving 1 vs. 2 spot guards. In the absence of any interaction, the specific guard clypeal pattern combination did not influence focal wasp choice, consistent with the idea that relative differences in signal intensity (i.e. brokenness, or number of spots), rather than specific patterns, are important to the receiver when assessing rivals (Tibbetts & Lindsay 2008).

The finding that clypeal patterns are not involved in rival assessment stands in contrast with that of Tibbetts & Lindsay (2008), who found evidence for status signalling in a North American population of the same species. In the same set-up as used in this study, Tibbetts & Lindsay (2008) demonstrated a strong preference for feeding near the low-status guard. In their study, preference for the low-status guard was observed regardless of the specific combinations of guard clypeal patterns, suggesting that focal wasps discriminated between rivals based on clypeal patterns in general, rather than showing preference for, or avoidance of, a particular pattern (Tibbetts & Lindsay 2008). Furthermore, in a second choice experiment, Tibbetts *et al*. (2010) again demonstrated a preference for feeding near the low-status guard, although this preference was found to be dependent on the clypeal pattern of the focal wasp (Tibbetts *et al*. 2010). Taken together, these findings suggest that, in North American populations, foundresses attend

to clypeal patterns during social interactions, and that behavioural responses are, at least in part, determined by asymmetries in clypeal patterning between rivals.

3.4.1 Methodological differences between studies

This study represents the first explicit test of status signalling in a population of *P. dominulus* in its native European range. Given the number of studies furnishing empirical support for status signalling in *P. dominulus* (Tibbetts & Dale 2004; Tibbetts 2008; Tibbetts & Lindsay 2008; Tibbetts & Izzo 2010; Tibbetts *et al*. 2010), it is perhaps surprising that I failed to find evidence for status signals operating in the Spanish population. The experimental set-up used for the choice trials closely resembles that used in previous studies by Tibbetts and colleagues. One difference, however, is the use of 0-spot focal wasps, rather than 1-spot wasps used by Tibbetts & Lindsay (2008). I used 0-spot wasps in order to maximise the sample size, as they represent the most common pattern type in the Spanish population . However, a potential concern with using 0-spot wasps rather than 1-spot wasps is that the two groups may behave differently if information about personal quality is also used in guard choice (Tibbetts *et al*. 2010). A previous study by Tibbetts *et al*. (2010) found that 0-spot wasps showed no preference for 1 vs. 2 spot guards: 0-spot guards are considered to be of low quality, and are argued not to choose between opponents of higher quality (1 and 2-spot guards). Although the 0-spot focal wasps used in the present study may not discriminate between 1 vs. 2 spot wasps for this reason, they would still be expected to prefer to challenge the 0 spot guard in the 0 vs. 1-spot and 0 vs. 2-spot guard combinations, as a 0-spot guard represents a more equal competitor (*i.e*. signals a similar RHP). The finding that focal wasps do not prefer to challenge the 0-spot guard when present suggests that the lack of preference is not the result of decisions based on information about individual quality, but rather is consistent with the absence of visual status signalling in this population.

The level of motivation experienced by the focal wasps is a second factor that may account for the difference between the results from this study and those from previous studies. Tibbetts (2008) has shown that when hunger levels are increased, thereby increasing the value of the food source, wasps are more likely to challenge a high-status guard. Could high hunger levels experienced by focal wasps account for the absence of

rival assessment in this study? Several aspects of the experimental design I used suggest that this is unlikely. Firstly, focal wasps used in the experiment were starved for 24 hours prior to the choice trials, the same starvation period experienced by wasps in Tibbetts & Lindsay's (2008) study. Prior to this period, wasps were provided with ad libitum sugar for 48 hours: food reserves were therefore unlikely to be low before the starvation period. Although Tibbetts (2008) found that motivation to challenge a guard increased with increasing hunger levels, this effect was only observed when comparing wasps starved for three days and wasps given unrestricted food. Wasps starved for 24 hours were not significantly more likely to approach the high-status guard than wasps that had not been starved, nor were they significantly quicker to approach the food source, or spend longer eating (Tibbetts 2008). Although the study of hunger levels and motivation to challenge used 1-spot focal wasps (Tibbetts 2008), there is currently no reason to believe that tolerance to starvation varies systematically with clypeal patterning, such that adults with more elaborate clypeal patterns are better able to withstand starvation. Indeed, the fact that, in this study, latency to eating was similar to that reported previously (median time to eating was 50s vs. 1 min in Tibbetts & Lindsay (2008)) suggests that focal wasps were similarly motivated when presented with the choice task. The fact that a significant proportion of wasps tested did not approach the sugar after the starvation period also indicates that hunger levels in focal wasps were not too high to preclude rival assessment.

Finally, a third possible reason for the absence of a preference between guard clypeal patterns in this experiment may have been that the focal wasps were unable to see the guards. However, in a second experiment, wasps showed a significant preference for eating at the unguarded versus guarded sugar cube. This result indicates wasps were able to see guards and supports the assumption underlying the choice paradigm that wasps should prefer to feed wherever the risk of competition from conspecifics is lowest. Overall, therefore, I am confident that this result is not an artefact arising from differences in experimental design. Rather, this result points to a genuine difference in signal function between American and European populations: clypeal patterns do not appear to represent signals of status in Spanish *P. dominulus*.

3.4.2 Population differences in status signalling

The negative result of this study is consistent with other observations on European populations of *P. dominulus* (Rusina *et al*. 2007; Cervo *et al*. 2008; Zanette & Field 2009). Cervo *et al*. (2008) found that the brokenness of the pattern did not differ significantly between the Rank 1 and Rank 2 foundresses in co-foundress groups. In a separate study, Zanette & Field (2009) explored the influence of clypeal patterns on social rank earlier in the pre-emergence phase of the colony cycle, before group stabilisation. Within co-foundress groups, the size of the clypeal pattern had a weakly significant effect on rank, with the Rank 1 sporting a larger black mark than her subordinates. Although cited as evidence for an association between clypeal patterning and dominance (Tibbetts & Shorter 2009), Zanette & Field's (2009) results should be interpreted with caution. First, the effect of clypeal patterning was no longer significant when other potential determinants of rank were included in the regression (Zanette & Field 2009). Second, the study measured only the size of the clypeal mark, and did not quantify pattern brokenness. Tibbetts (2010) has argued that the area of the pattern represents the non-signalling component, while the active signalling component is the pattern's brokenness. A (weak) association between pattern size and rank would therefore say little about the potential for status signalling in the Spanish population. Indeed, among patterned wasps in this population, area and brokenness of the clypeal pattern are not significantly correlated (J. P. Green, unpubl. data).

A striking feature of the Mediterranean populations is that clypeal pattern variability is much lower than has been reported for American populations. While around 80% of wasps in the population investigated by Tibbetts & Dale (2004) have black clypeal patterns, the proportion of wasps with clypeal patterns is only 40% in Italy (Cervo *et al*. 2008) and as low as 20% in Spain (see Chapter 5). An important consequence of this low variability in clypeal patterning is that its importance in mediating social conflict is necessarily limited. The presence of clypeal patterning is neither necessary nor sufficient for the acquisition of high rank, as demonstrated by Cervo *et al*. (2008) and Zanette & Field (2009). Furthermore, the majority of contests over resources such as food or reproduction will be between individuals without clypeal marks. Rival assessment based on clypeal patterning will therefore often fail to reveal differences in

individual RHP in these populations. I suggest that the difference in prevalence of clypeal patterns between Mediterranean and American populations can be considered further evidence of genuine variation in signal use between these populations.

Geographic variation in the value of secondary sexual traits is well documented (for a review see Wilczynski and Ryan 1999). In particular, variation in the signal function of avian plumage has received considerable attention (e.g. Price 2006; Dunn *et al*. 2008; Takahashi *et al*. 2008). However, most studies have focused on the value of intersexual traits (i.e. ornaments used in mate choice), while there are few accounts of variation in the value of intrasexual traits, including status signals. One possible example of population divergence in status signalling comes from studies on the collared flycatcher in two sites in Europe. In Sweden, the forehead patch acts as a signal of status in territorial contests between males (Pärt & Qvarnström 1997; Qvarnström 1997), whereas, in Hungary, territory defence is not associated with forehead patch size (Garamszegi *et al*. 2006). The reason for this variation in plumage function is not currently understood, but in the collared flycatcher there is some evidence that the forehead patch is also a target of female choice (Gustafsson *et al*. 1995). In cases where status signals are additionally used in mate choice, variation in the signal phenotype could be explained by divergence in female preference for the trait, rather than changes in the intensity of intrasexual competition between populations.

In *P. dominulus*, clypeal patterns are present only on females, and there is no evidence from observations of mating behaviour that males choose among females (Beani 1996). What then might account for variation in the frequency of clypeal patterns and their use as signals between populations? One factor promoting variation may be genetic drift, with populations passing through genetic bottlenecks during the first founding events in North America 30-40 years ago (Cervo *et al*. 2000). Evidence by Tibbetts (2010) that the amount of black on the clypeus is heritable indicates that an initial differences in pattern prevalance may be preserved or even amplified over generations. However, the recent finding of relatively high genetic variability in American populations points to multiple independent founding events (Liebert *et al*. 2006), suggesting that bottlenecks may not have been particularly severe. Another explanation could be that variation in pattern prevalence and function is due to environmental effects on the development of

the clypeal pattern. Under the developmental conditions present in some environments, clypeal patterning may be prevalent/variable enough within the population to function as a status signal, while conditions in other environments result in less variable patterns. In particular, there is evidence that climatic factors, such as temperature and humidity, affect the extent of melanin patterning in insects (Usui *et al*. 2008; Parkash *et al*. 2008), including *Polistes* wasps (Enteman 1904). Climate differences may therefore explain inter-population variation in *P. dominulus* clypeal patterns, with lower temperatures and/or higher humidity generating more variable clypeal patterns in populations at higher latitudes, as is seen in a number of bird species (Price 2006). This hypothesis is consistent with the greater pattern variability in the northeastern United States than in the Spanish population. Data on pattern variability and status signalling from other, more northerly, populations in the species' ancestral range may provide a clearer picture of possible climatic effects on the development and signal function of clypeal patterns.

2.5 Summary

Research on North American populations of *P. dominulus* has indicated that clypeal patterns function as a signal of status during competition. However, data from studies in *P. dominulus*' native European range have failed to find a strong link between clypeal patterning and RHP. I found that wasps in a Spanish population do not use clypeal patterns to assess rivals in a competitive context. Variation in signal use between North America and Spain is associated with differences in clypeal pattern variability at the population level. Genetic drift appears unlikely to account for these differences; rather, variation in Environmental factors such as climate may drive divergence of patterns between populations, a hypothesis which I explore in Chapter 5.

In this chapter I focused on the possible signal value of *P. dominulus* clypeal patterns in a Spanish population. Although I found that clypeal patterns are not used in rival assessment, the extent to which the patterns are correlated with RHP or other aspects of quality is unknown. In the following chapter (Chapter 4), I explore the relationship between clypeal patterning and various measures of fitness in a natural population of *P. dominulus* to determine whether the clypeal pattern reflects quality in the wild.

Chapter 4: The quality basis of clypeal patterning in *Polistes dominulus*

4.1 Introduction

4.1.1 The adaptive value of sexually-selected traits

Quantifying the adaptive value of traits is a central goal in behavioural ecology. A trait can be considered to have adaptive value if, in a given environment, its presence enhances fitness relative to that of a variant lacking the trait (Reeve & Sherman 1993). Although fitness has been formalised in a number of ways (Hunt & Hodgson 2010), a common definition is the number of descendents that an individual produces relative to the average number of descendents produced by other individuals in the population (Hunt *et al*. 2004). In practice, the fitness of an individual is often estimated as its lifetime reproductive success (LRS), that is, the number of offspring it produces during its lifetime (Grafen 1988). Broadly speaking, LRS is the product of individual survival and reproduction (Newton 1989); adaptive traits are therefore those that promote individual survival and/or reproduction.

Adaptations for survival are considered to have evolved by natural selection. In contrast, many of the adaptations for reproduction have evolved by the process of sexual selection. Sexually-selected traits have traditionally been divided into two main types: intersexual traits, which aid in reproduction by attracting mates, and intrasexual traits, which function in competition with members of the same sex, either for direct access to reproduction, or else for access to resources (e.g. territories) that boost reproduction (Andersson 1994). Research investigating the adaptive value of such traits has generally proceeded in three distinct phases. First, observational studies have sought to demonstrate correlations between the intensity of a trait and reproductive success (e.g. Siefferman & Hill 2003; Jensen *et al*. 2004). Second, where positive correlations are found between trait intensity and reproductive success, experimental manipulations of the trait have been performed to test the causality of the association (e.g. Veiga 1993; Petrie & Halliday 1994; Stapley & Keogh 2006). Third, where the impact of a trait on reproductive success has been demonstrated experimentally, an adaptive explanation is sought (e.g. the trait functions as a signal of quality to mates or rivals) (Andersson

1994). Thus, a trait may properly be considered an adaptation that has evolved via sexual selection only in the event that a positive effect of the trait on (lifetime) reproductive success is demonstrated.

4.1.2 Identifying traits under intrasexual selection

The evolution of intersexual traits has long been a topic of vigorous debate, due in part to difficulties in identifying the benefits that are gained from mate choice based on (exaggerated) ornaments. In contrast, the role of intrasexual traits in determining an animal's resource-holding potential (RHP) is often intuitive. For instance, structures such as horns and tusks function as weapons in physical combat, while cowering postures may be adopted to indicate submission during a contest (Maynard Smith & Harper 2003). In some cases, however, the outcome of contests appears to be determined by asymmetries in traits that bear no obvious relation to RHP. In particular, in a number of species, the likelihood of winning a contest is predicted by differences in body coloration between contestants, with individuals sporting larger or brighter colour patches being more likely to secure the contested resource (Senar 1999; Whiting *et al*. 2003; Chapter 1). Furthermore, the outcome of such contests can have an impact on reproductive success (e.g. via the attainment of breeding territories), and positive correlations between coloration and reproduction have been reported for some species (Whiting *et al*. 2003, and references therein). To test whether differences in coloration are directly responsible for variation in resource acquisition among individuals, experimental manipulations of body coloration have also been attempted. In some cases, such manipulations are seen to result in changes in an individual's success during contests (e.g. Qvarnström 1997; Whiting *et al*. 2003). Colour patterns in these species would thus appear to represent an adaptation to competition, and have been referred to as conventional signals or status signals (Maynard Smith & Harper 1988).

4.1.3 The adaptive value of clypeal patterning in *Polistes dominulus*

Behavioural observations coupled with experimental manipulations have uncovered evidence for status signalling across a range of taxa (see Chapter 1). In all cases, coloration has first been shown to predict RHP in the wild, and has then been shown to communicate RHP to rivals (reviewed in Senar 1999). In this chapter, I focus on what

may be the single exception – the status signal in the paper wasp *Polistes dominulus*. In *P. dominulus*, variable black patterns on the clypeus have been argued to function as status signals during competition among females (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). In North American populations, the outcome of lab-based contests is predicted by the disruption (brokenness) of the clypeal patterns (Tibbetts & Dale 2004). Furthermore, experimental manipulations of the patterns result in changes in the dynamics of contests that are broadly consistent with predictions of status signalling (Tibbetts & Dale 2004; Tibbetts & Izzo 2010; Chapter 1). Based on these findings, clypeal patterning in *P. dominulus* have been interpreted as an adaptation for competition, reducing the risk of escalated conflict during contests (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010; Tibbetts & Izzo 2010).

In the last decade, research on status signalling in *P. dominulus* has helped to revive the concept of status signalling, by offering potential resolutions to a number of theoretical difficulties, chief among them the seeming paradox of how signals can be reliable if they cost little to produce (Tibbetts & Izzo 2010; Chapter 1). However, as I argue below, there are strong grounds for questioning whether an adaptive explanation has not been arrived at prematurely. The fundamental concern is that, as yet, we have no evidence about how clypeal patterning is related to fitness in the wild. In the lab, interactions occurring in the absence of a fitness-enhancing resource point to a competitive advantage for individuals with highly broken patterns (Tibbetts & Dale 2004). However, for brokenness to have adaptive value, it must also be shown that individuals with greater brokenness enjoy greater fitness pay-offs than those with a less broken phenotype. Surprisingly, this has not been explored in any significant detail in the American populations. In further lab experiments, Tibbetts & Shorter (2009) found that brokenness predicted success in nest usurpation, one strategy by which wasps can secure resources and access to reproduction (Reeve 1991). Tibbetts *et al.* (2011b) have also shown that brokenness predicts time of emergence from diapause under lab conditions, and there is some suggestion that early-emerging females enjoy a competitive advantage in nest founding (Röseler 1991). However, no studies have shown that brokenness is positively correlated with fitness (or a proxy such as LRS) under natural conditions. Determining the relationship between brokenness and fitness in the wild not only represents a vital step in quantifying the adaptive value of clypeal

patterns, but is also important if we are to understand the apparent divergence in signal value between different populations (Green & Field 2011b; Chapter 3). In European populations of *P. dominulus*, clypeal patterns do not appear to signal RHP during contests (Green & Field 2011b; Chapter 3), and there is no relationship between brokenness and rank in co-foundress groups (Cervo *et al*. 2008). However, as yet, there has been no attempt to determine the relationship between clypeal patterning and fitness in the European populations.

4.1.4 Estimating fitness in *P. dominulus*

In this chapter, I aim to explore the relationship between clypeal pattern brokenness and fitness in a Spanish population of *P. dominulus* under field conditions. As noted above, LRS is a commonly-used proxy for fitness in studies of wild animal populations. LRS can be viewed as a product of an individual's survival (in terms of the number of breeding seasons in its lifetime) and its reproductive output (the number of broods and the number of young/brood) in each breeding season (Newton 1988). For vertebrates, breeding lifespan is often the most important determinant of LRS (Clutton-Brock 1988); however, the relative longevity of many vertebrates makes it difficult to track individual success over each breeding season. In contrast, LRS can be more easily quantified in *P. dominulus*[5], whose reproductive output is limited to a single nesting season. On the other hand, the relatively complex social system of *P. dominulus* compared with that of many vertebrates presents a number of challenges when exploring the relationship between traits and fitness measures. *P. dominulus* is a primitively eusocial species that exhibits considerable flexibility in behavioural and reproductive strategies (Reeve 1991). Mated females emerge from diapause in the spring and found colonies, either singly or, more commonly in the Spanish population, in small groups. Reproduction by singly-nesting females (lone foundresses) is a product of survival and fecundity; however, reproduction in co-foundresses group is determined in the first instance by hierarchical rank in the group, with the dominant producing the vast majority of offspring (Queller *et al*. 2000; Leadbeater *et al*. 2011). Consequently, co-foundress

[5] Reproduction does not represent the only source of fitness in *P. dominulus*: the opportunity to help relatives means that indirect (kin-selected) benefits also contribute to an individual's overall (inclusive) fitness. The aim of this study was to quantify direct benefits (i.e. through reproduction), and indirect benefits were not measured. The reproductive success data I present in this study therefore provide an estimate of direct fitness, but not of inclusive fitness

reproduction will depend not only on individual attributes (survival and fecundity) but also on group attributes, specifically the relative RHP of other group members, which is thought to play a role in shaping the initial hierarchy (Röseler 1991).

Estimates of foundress LRS are also complicated by the change in the reproductive value of offspring across the season (Leadbeater *et al*. 2011). Although all *P. dominulus* females are capable of mating, the majority of female offspring produced earlier in the season do not reproduce but instead remain on the nest as workers. In contrast, the majority of late-emerging females mate and overwinter to found colonies the following spring (Reeve 1991; Leadbeater *et al*. 2011). In estimating LRS, it is therefore necessary to consider both the number of offspring produced and the time in the season in which they are produced. Adopting a season-wide view of reproduction is also important if the reproductive output of subordinate foundresses is to be quantified accurately. Although the original dominant foundress in a group produces the majority of offspring, her position in the hierarchy may be inherited upon her death, allowing former subordinates the opportunity to reproduce (Leadbeater *et al*. 2011). Indeed, inheritance of the dominant position has been shown to account for the majority of reproduction enjoyed by subordinates and occurs most commonly later in the season, after worker emergence (Leadbeater *et al*. 2011).

In this study, I examined female (foundress) reproduction across the entire nesting season in relation to the brokenness of the clypeal pattern (males are not considered as they do not have variable clypeal patterns). Due to practical considerations[6], it was not possible to track reproduction by individual foundresses across their natural lifetimes, (LRS). Instead, reproductive success (RS) was estimated during each of five contiguous time periods spanning the entire nesting season. While a positive correlation between RS and brokenness would provide strong support for brokenness being an adaptation, it would not reveal *how* brokenness impacts on fitness (Grafen 1988). Numerous aspects of an individual's quality contribute to its fitness, with complex interactions and trade-

[6]The data presented here were collected as part of a larger study aimed at estimating the frequency of nest inheritance by subordinate foundresses over the season (see Leadbeater *et al*. 2011). To estimate rates of inheritance, subordinate reproduction was quantified at several time points in the season by collecting subsets of nests and genotyping foundresses and brood. The need to collect nests meant that it was not possible to quantify an individual foundress's reproductive success over its natural lifetime (i.e. its LRS).

offs between individual quality components (Hunt *et al*. 2004). In order to determine the contribution of brokenness to fitness in more detail, I also explored the relationship between other components of individual quality and brokenness.

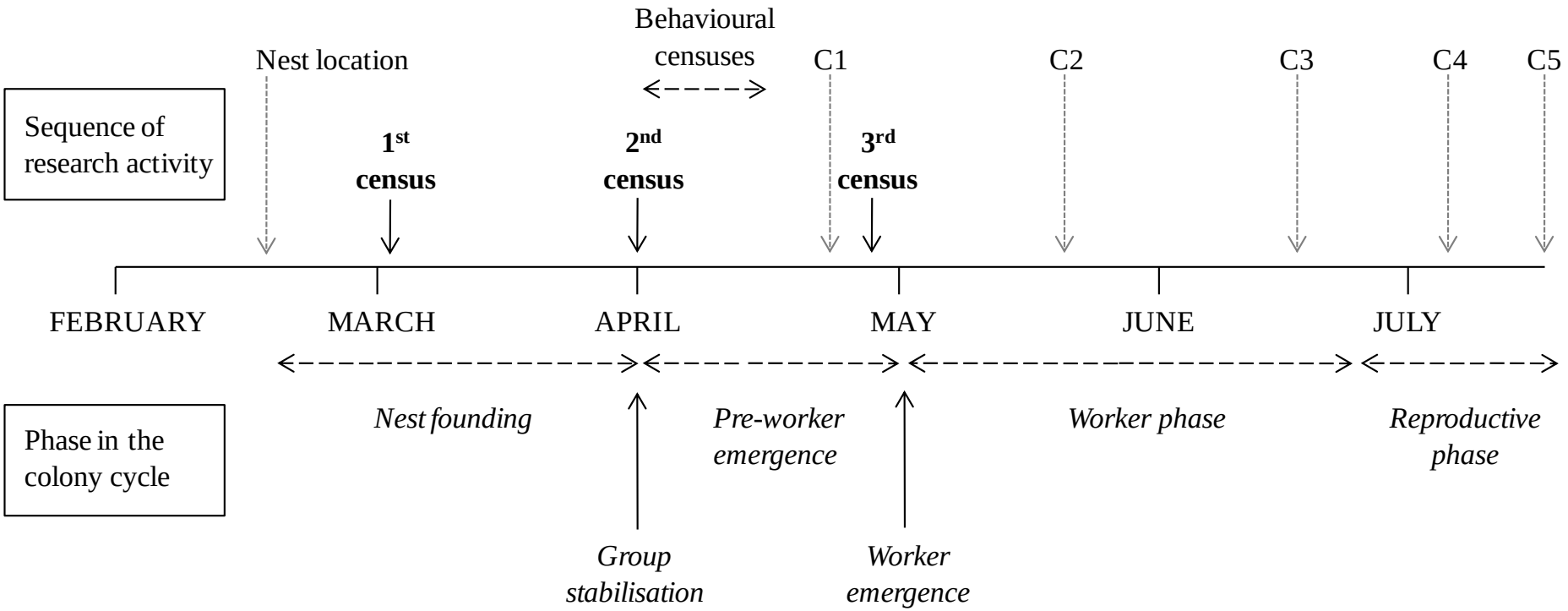
Evidence from previous research indicates that brokenness is most likely to reflect aspects of quality relating to RHP (Tibbetts & Dale 2004; Tibbetts & Shorter 2009; Tibbetts *et al*. 2011a). RHP itself is a complex and multifaceted trait (Parker 1974), and is poorly understood in *P. dominulus*. Potential determinants may include body size (Tibbetts & Shorter 2009; Green & Field 2011a; Chapter 6; but see Cant *et al*. 2006) and juvenile hormone titre (Tibbetts *et al*. 2011a). Consequently, when testing for an association between brokenness and RHP in the wild, it is more straightforward to look for correlations between brokenness and the pay-offs that individuals gain through competition. Therefore, as well as looking at reproduction in co-foundress groups, I also explored the relationship between brokenness and rank in the hierarchy, which is closely linked to reproduction and determined through competition between co-foundresses (Reeve 1991). Brokenness has also been linked to individual condition (Tibbetts & Curtis 2007). I therefore also tested for associations between brokenness and (a) survival in the wild and (b) nest-joining strategies, which may reflect individual condition (Starks 2001). Overall, then, the aim of this study was to quantify the effect of clypeal pattern brokenness on measures of fitness in a natural population of *P. dominulus*, and, in doing so, to determine the adaptive value of brokenness in the wild.

4.2 Materials and Methods

4.2.1 General methods

A summary of the main field methods used is provided in Figure 4.1 (overleaf). All statistical analyses were performed in R. Means are reported ± 1 s.e., unless otherwise stated.

Figure 4.1. Sequence of field methods used in relation to timing of events in the colony cycle. The three morning censuses (in bold) are when foundresses were painted, photographed and tarsal-sampled. C1-C5 show the 5 nest collection times. Timing of colony events are approximate, and vary from nest to nest depending on age and development. Offspring production is split into a worker phase and a reproductive phase based on data from Leadbeater *et al*. (2011).



Study population and nest censuses

P. dominulus foundresses were studied at three nearby agricultural sites close to Conil de la Frontera (Cádiz Province, Spain) from February – July 2008. Detailed timings of events in the colony cycle in the Spanish populations can be found in Chapter 2 (section 2.2.1). Nests were located during the nest-founding phase in late February – early March and were subsequently monitored throughout the entire nesting season until dispersal of reproductives in mid-July. At the start of nest founding, a census of all nests was carried out between approximately 0500-0800 when all foundresses were present on the nest and were inactive. All foundresses were collected and given a unique paint mark on the thorax using Humbrol enamel paint. During marking, a photograph was taken of the face of every foundress that had clypeal patterning (see *Morphological Measurements*). Additionally, for each foundress, the tarsus from one of the middle legs was clipped with scissors and stored in 1ml pure ethanol for use in genotype sequencing. Tarsal sampling has been used in other studies (Liebert *et al*. 2005; Leadbeater *et al*. 2010) and allows a foundress's reproductive success to be estimated in the event of her death or disappearance during the season. Two further morning censuses were carried out in late March at group stabilisation and late April at worker emergence, during which any new foundresses were paint-marked, tarsal-sampled and photographed (where clypeal patterns were present). A total of three morning censuses were thus performed, in which all foundresses on the nest were recorded: one at nest founding, a second at group stabilisation, and a third and final census one month later, at worker emergence (see Figure 4.1).

Morphological Measurements

I took photographs of all foundresses with clypeal patterns during foundress marking. Wasps were held still and photographs of the clypeus were taken using a Nikon D80 digital camera (with macro lens) from a fixed distance and under relatively constant lighting conditions. Image analysis was then used to calculate the brokenness of the clypeal pattern (for details see Chapter 2). Multiple photographs were taken from a sample of 24 individuals to assess the reliability of pattern measurements in the field. Repeatability, calculated using the concordance correlation coefficient (Zar 1996), was high (r = 0.96, 95% CI: 0.91-0.98). In several analyses, I use the presence/absence of

clypeal patterns as a simple proxy for brokenness, following Tibbetts & Lindsay (2008). In general, individuals with clypeal patterns have a higher brokenness than those without patterns (see Chapter 1).

While the primary focus of the study is clypeal pattern brokenness, I also explore the role of size in foundress quality. Although several studies indicate that body size predicts RHP during usurpation contests (Tibbetts & Shorter 2009; Green & Field 2011a; Chapter 6), attempts to assess the importance of body size in the wild have met with mixed results: in some populations, hierarchical rank is positively correlated with size (Cervo *et al*. 2008), while in others it is not (Zanette & Field 2009). However, the relationship between size and reproductive success remains to be explored. In this study, I include size as a covariate in order to determine the effect of size on rank, reproduction and survival in the Spanish population during the 2008 season. The width of the first metatarsus at the widest point, measured under a dissecting microscope at 64× magnification, was used to estimate body size for each foundress. Tarsus width was positively correlated with head width (Pearson's $r = 0.5$), a commonly-used measure of body size in other studies (e.g. Tibbetts & Dale 2004; Cini *et al*. 2011).

4.2.2 Survival and reproductive success of lone foundresses

Methods

Lone foundress and co-foundress RS was examined separately. Lone nesting represents a relatively rare strategy in this population, and is associated with a high failure rate early in the season (Leadbeater *et al*. 2011). I was nonetheless able to collect data for 56 females, identified as lone foundresses by the morning census undertaken at group stabilisation. I estimated reproductive output for these females by counting the number of pupae present in cells immediately prior to worker emergence. In cases where nests did not survive to worker emergence, I recorded a pupal count of zero. Because pupae on surviving nests were not genotyped, the possibility exists that a proportion of brood was produced by a wasp other than the marked foundress. However, censuses of these nests showed that the marked foundress was the only wasp present at group stabilisation and at worker emergence. Furthermore, data on reproductive skew in two-foundress

groups in this population indicate that subordinates receive little or no reproduction (Leadbeater *et al*. 2011). Therefore, the pupae counted for each colony are likely to the offspring of the marked foundress, rather than the offspring of an unmarked wasp briefly arriving at/joining the nest.

Statistical Analyses

RS for lone foundresses was estimated by pupal counts, In general, RS was very low. Forty-seven of the 56 foundresses produced no pupae, and the remaining 9 foundresses produced between 1-12 pupae (mean = 4.33 ± 1.22). The high number of zero counts resulted in zero-inflation of the data, which I therefore analysed using a GLM with a zero-inflated, negative binomial error structure ('ZINB' model; Zuur *et al*. 2009b). The ZINB model assumes that the zero counts are the result of two distinct processes: failure of the nest to survive to the pupal stage, and failure of those nests that survive to produce any pupae. The probability that a nest survives to the pupal stage is predicted by a binomial distribution, while the number of pupae that surviving nests produce is predicted using a negative binomial distribution, allowing for zero counts from these nests. Model comparisons based on Vuong's non-nested test (Zuur *et al*. 2009b) indicated that a negative binomial error structure was a better fit to the data than a Poisson structure. Only a small number of lone foundresses (14) had clypeal patterns. In the analysis, I therefore used presence/absence of clypeal patterns as a simple proxy for brokenness. I fitted the presence/absence of patterns, body size and site as explanatory variables in the binomial part of the model, and presence/absence of patterns and size as explanatory variables in the negative binomial part of the model.

4.2.3 Changes in group composition: survival and late-joining

Methods

The initial phase of the colony cycle is characterised by high rates of foundress movement between nests, resulting in frequent changes in group membership (Zanette & Field 2011). As the rate of movement falls, group membership stabilises, with fluctuations in group size then largely the result of foundress mortality. However, a small proportion of wasps continue to join groups into the late pre-emergence phase of

the colony cycle. These late-joiners may be pursuing a 'sit-and-wait' strategy, where energy that would otherwise be spent on nest founding is conserved for reproduction on established nests (Starks 2001). Therefore, the time at which an individual joins a nest could provide important information about that individual's quality.

To see firstly whether the proportion of wasps with clypeal patterns changed over the season, either as a result of differential survival and/or alternative joining strategies, I used data from the three morning censuses to compare the ratio of wasps with patterns to wasps without patterns in 146 co-foundress groups at three times over a two-month period: early March at nest founding; late March at group stabilisation, and late April in the late pre-emergence phase. To look in more detail at the factors responsible for any change in the numbers of wasps with and without patterns over the season, I also recorded the survival of 118 foundresses in 22 co-foundress groups, in which ≥ 1 foundress had clypeal patterns (2-13 foundresses/group; mean group size = 5.36 ± 0.60 foundresses). Survival over a one-month period following group stabilisation was assessed by recording whether foundresses present at the second morning census in late March were still on the nest by the third morning census in late April. Finally, to see whether clypeal patterning was associated with late-joining, I compared the clypeal patterns of wasps marked at the third morning census (i.e. those joining after group stabilisation) with those of foundresses marked at the second morning census at group stabilisation in a further 29 groups where late-joining was observed (excluding marked wasps arriving from other known nests).

Statistical Analyses

First, to see whether wasps with clypeal patterns were more prevalent on nests at certain times of the season, I compared the ratio of wasps with patterns to wasps without patterns at three time points in the season using Cochran's Q test. Cochran's Q test compares non-independent sets of frequencies or proportions, and can here be used to test for differences in the proportion of wasps with clypeal patterns on the same nest over different time points. Second, to test whether foundress survival was related to clypeal patterning, I used a GLMM with binomial errors with survival (yes or no) as the binary response variable. To control for similar conditions experienced by foundresses

in the same group I fitted 'nest' as a random factor in the analysis. Clypeal pattern brokenness, body size and site were included as explanatory variables. Due to the high rates of disappearance and nest abandonment among co-foundress groups at Site 3, I chose to analyse survival of foundresses at sites 1 and 2 only. Third, to test whether wasps with clypeal patterns were more likely to join nests later in the season, I compared the proportions of wasps with clypeal patterns that were marked before and after group stabilisation using Pearson's χ^2 test. I also compared pattern brokenness between wasps with patterns joining before and after group stabilisation using a Mann-Whitney U test. Finally, to see whether late-joining wasps differed from other members of their group with respect to clypeal patterning, I compared the brokenness of late-joiners with the mean brokenness of wasps already established in the group using a Wilcoxon signed-rank test.

4.2.4 Behavioural dominance in co-foundress groups

Methods

A division of labour is commonly observed in *Polistes* co-foundress groups, with some individuals engaging in activities such as foraging, defence and brood care at a greater frequency than others (Reeve 1991). Moreover, the propensity to engage in cooperative behaviours varies with inheritance rank, with individuals of lower rank spending a greater time provisioning and defending the colony than more higher ranking individuals (Cant & Field 2001). The amount of time an individual spends away from the nest provides an estimate of its foraging effort, and can thus be used as a rough proxy for its rank within the hierarchy (Cant & Field 2001; Leadbeater *et al*. 2010). By recording the proportion of day-time censuses in which individuals were off the nest, I was able to infer the rank of 97 foundresses in 18 co-foundress groups in which ≥ 1 foundress had clypeal patterns (2-14 foundresses/group; mean group size = 5.39 ± 0.64 foundresses). Censuses of co-foundress groups were performed between 1100-1600 on sunny days when wasps were able to forage. For each group, a minimum period of 45 minutes separated each census. Censuses were performed for a minimum of two consecutive days (mean = 11.77 ± 0.67 censuses per nest) in the two weeks following the second morning census at group stabilisation.

Statistical Analysis

The behavioural data were analysed in two ways, with procedures following those of Zanette & Field (2009). First, for each individual, I estimated foraging effort as the proportion of censuses in which that individual was away from the nest. Within a group, the amount of time spent by each wasp foraging is unlikely to be independent of the activities of its nest-mates. For example, a wasp's decision to forage might be sensitive to the current need for nest defence, which will be higher when a greater proportion of its nest-mates are off the nest foraging. In order to examine the influence of brokenness on foraging effort, I therefore compared the observed mean within-nest correlation between brokenness and foraging effort with that obtained using simulated groups in which the proportion of time off the nest was randomised. To begin with, I calculated Spearman's rank correlation (ρ) between brokenness and foraging effort for each group, and then calculated the overall observed mean correlation across groups. I then recalculated ρ after foraging effort was randomly permuted in each group, to obtain an overall simulated mean correlation. I repeated this simulation procedure 10 000 times to obtain a null distribution of means. The proportion of simulated means equal to or stronger than the observed mean was used as an estimate of the probability (p) of obtaining the observed correlation by chance. Correlations were considered to be significant if $p < 0.05$. The same procedure was then used to evaluate the relationship between foraging effort and body size.

Because size and brokenness are examined separately, the above analysis does not control for any correlations between brokenness and body size or for effects of group size and site. I therefore analysed the combined effect of these variables on foraging effort in a GLM, using quasibinomial errors to account for overdispersion in the data. Because foraging efforts of individuals in a group are not independent, I sampled 1 foundress at random from each group. Proportion of time off the nest was the response variable and brokenness, body size, group size and site were fitted as explanatory variables. Hierarchical rank is likely to be determined by asymmetries in RHP between co-foundresses (see Introduction); in this analysis, I therefore calculated brokenness and size relative to that of other co-foundresses in the group. Relative brokenness was calculated as a foundress's brokenness divided by the mean brokenness of all co-

foundresses in the group, and relative size was calculated in the same way. I repeated the procedure of sampling followed by GLM 2000 times in order to determine how frequently significant results were obtained ($\alpha < 0.05$). In the absence of an effect of the variable of interest, a significant result would still be expected in 5% cases. I considered explanatory terms to be significant if their associated *p* values < 0.05 in at least 5% of the re-sampling runs.

4.2.5 Reproductive success in co-foundress groups

Field Methods

RS was estimated for 252 co-foundresses from 38 groups in which ≥ 1 foundress had clypeal patterns. To explore the effect of brokenness on reproduction across the entire season, nest collections were made at five points over the season. At each time point, 6-9 nests were randomly chosen from all surviving nests in the population (ignoring any nests found to be parasitised by the social parasite *Polistes semenowi*). Nests (plus associated wasps) were collected at night and stored at -80°C at the University of Cádiz before being transported to the UK for genetic analysis. Nest collections were timed based on the development time of brood on the nest. Development times were estimated by mapping the brood on a subset of nests, updating estimates a further two times as the season progressed. The first sample of nests was collected in late April; pupae on these nests represented the first offspring to be produced in the season. Subsequent nest collections were timed so that the oldest pupae on collected nests had pupated immediately following the previous collection date. Nest collections were made on the following dates, (the phase of the colony cycle in which pupae were originally produced is indicated in parenthesis): (1) 23rd April (nest founding), (2) 21st May (pre-worker emergence), (3) 18th June (worker emergence), (4) 4th July (late worker – reproductive phase) and (5) 17th July (reproductive phase). In all, a total of 38 nests were collected.

In the analyses of co-foundress RS, I again used relative values of brokenness and size (calculated as in section 4.2.4). In order to derive meaningful relative values for each co-foundress, it was important that all co-foundresses sampled were in the group at the same time (and so were at least capable of competing for dominance/reproduction).

Because the groups were not censused daily, I did not have information about the precise fluctuations in group membership through the season. Consequently, in the analysis, I decided to include all foundresses present on the nest at the second morning census for nests in Collection 1, and all foundresses present at both the second and third morning census for nests in Collections 2-5. By selecting only foundresses recorded at these censuses, I could be confident that the relative trait values calculated for each co-foundress reflect the differences in traits between that foundress and other members of her group who were present during her tenure on the nest.

Laboratory Methods

RS was estimated by counting the number of pupae produced by each foundress in a group. Foundresses and pupae were genotyped at 9 microsatellite loci (see Chapter 2, section 2.3.1). Foundress DNA was extracted from tarsal samples collected during morning censuses. Any unmarked foundresses collected with the nest (i.e. foundresses joining after the last morning census) were also genotyped. All pupae on nests were genotyped, except for nests containing >30 pupae at collection, for which a random sample of 30 pupae were genotyped. Laboratory procedures are described in detail in Chapter 2 (section 2.3). Tests for linkage disequilibrium, deviation from Hardy-Weinberg Equilibrium, and heterozygote deficiency have previously been performed for the 9 microsatellites in a sample of *P. dominulus* females collected from the Spanish population in 2008-09 (Leadbeater *et al*. 2010, 2011). In each case, no significant deviation from chance expectation was reported (Leadbeater *et al*. 2010, 2011).

Maternity assignment procedures

Maternity assignment was performed using the software KINGROUP version 2.9 (Konovalov *et al*. 2004). To assign offspring to a particular individual (mother) in the group, I first used the genetic data to determine the sex of the offspring. In the Hymenoptera, females are diploid, while males are haploid. I therefore classed individuals that were homozygous at every locus as males, whereas I classed those that were heterozygous at one or more loci as females. Given the observed heterozygosities at the 9 loci, I calculated the probability of a female being homozygous at all 8 loci (and therefore of being wrongly identified as a male) to be 9.94×10^{-8}. I used the Full

Sibship Reconstruction procedure to partition female offspring into full-sister groups. Beginning with a single pair of offspring from the same nest, the procedure compares the hypothesis that the pair of individuals are full sisters with the hypothesis that they are cousins or unrelated by calculating the probability of the pair's genotypes occurring in each case, given the population allele frequencies. A third offspring is then added and the program proceeds to determine the most likely current grouping based on a comparison of alternative hypotheses of the relationship (sister or cousin) between the new offspring and each of the existing pair of offspring. Using this maximum-likelihood approach, the program continues to assign individuals to sister groups until all offspring have been introduced. Within a group of full-sisters, each individual will share a paternally-derived allele and, as a group, will possess no more than two maternally-derived alleles, which can be used to identify the mother among the adults on the nest. Since multiple mating in *P. dominulus* is thought to be rare (Queller *et al*. 2000; Strassmann 2001), I assumed single mating for all potential mothers included in the analysis (meaning that no individual could be the mother of more than one sister group). The maternity of male offspring was determined individually for each male by comparing its genotype with those of the adults on the nest.

Genotypes were obtained for a total of 514 female and 229 male pupae. Of the female pupae genotyped, I was unable to assign 11 individuals (1.83%) to an individual foundress, on account of alleles shared with ≥ 1 foundress in the group. Of the male pupae genotyped, I was unable to assign 83 individuals (36.24%) to an individual foundress. If males with genotypes common to more than one foundress in a group are excluded, male offspring production will consequently be biased towards those foundresses with rarer genotypes (i.e. those more distantly related to other group members). For this reason, I decided to exclude all male offspring from the analyses of co-foundress RS. RS was thus estimated as the number of female offspring (pupae) produced by each co-foundress in a group.

Statistical Analyses

As in the previous section, analysis of factors influencing reproductive success involved both randomisation tests and GLM. To quantify co-foundress RS, I recorded the number of female pupae produced by each co-foundress. Reproduction in co-foundress groups is likely to be zero-sum game. That is, when one individual produces an offspring, there is one fewer offspring available for production by other individuals. For this reason, offspring produced by individuals within a group cannot be considered as independent data points. Therefore, in order to test the relationship between brokenness and offspring production, I compared the observed mean within-nest correlation between brokenness and offspring number with that obtained using simulated groups in which offspring number was randomised. To begin with, I calculated Spearman's rank correlation (ρ) between brokenness and offspring number for each group, and then calculated the overall observed mean correlation across groups. I then recalculated ρ after offspring number was randomly permuted in each group, to obtain an overall simulated mean correlation. I repeated this simulation procedure 10 000 times to obtain a null distribution of means. . The proportion of simulated means equal to or stronger than the observed mean was used as an estimate of the probability (p) of obtaining the observed correlation by chance. Correlations were considered to be significant if $p < 0.05$. The same procedure was then used to evaluate the relationship between offspring number and body size.

As in the analysis of behavioural dominance, I complemented the above simulations with GLMs in which brokenness and size were considered together in the presence of other potential covariates. In order to explore factors affecting co-foundress RS, I separated the analysis into two steps: factors affecting whether or not a co-foundress produces offspring, and factors affecting how many offspring are produced among foundresses who succeed in producing offspring. In the majority (33/38) of co-foundress groups, only a single wasp produced offspring; however, in 5 groups, more than one foundress produced offspring. In the first step of the analysis, I tested whether brokenness and/or size affected the likelihood of producing pupae, using a GLM with binomial errors. Because reproduction of individuals in a group is not independent, I sampled 1 foundress at random from each group. Whether or not offspring were

produced was the binary response variable and brokenness, size and group size were fitted as explanatory variables. To test whether the importance of brokenness and size varies over the season, I also included interactions between brokenness and collection date, and size and collection date, as additional explanatory variables. The opportunity for reproduction is closely tied to hierarchical rank, which is determined by RHP differences among co-foundresses. Therefore, as in the analysis of behavioural dominance, foundress brokenness and size were calculated relative to other co-foundresses. I repeated the procedure of sampling followed by GLM 2000 times in order to determine how frequently significant results were obtained at the 95% confidence interval. As before, explanatory terms were considered to be significant if their associated *p* values were smaller than 0.05 in at least 5% of the re-sampling runs.

In the second step, I examined the factors accounting for variation in the number of offspring produced among wasps that successfully produced offspring. In the majority (33/38) of co-foundress groups, only a single wasp produced offspring; however, in 5 groups, more than one foundress produced offspring. To avoid the issue of non-independence of offspring counts for foundresses in the same group, I included only the most productive foundress from each group (the dominant reproductive) in the analysis. I explored factors affecting the number of offspring produced in a GLM, using quasipoisson errors to account for overdispersion in the data. Offspring number was fitted as the response variable, and brokenness and size were fitted as explanatory variables, together with group size, site and collection date. As before, I also fitted interactions between brokenness and collection date and size and collection date as additional explanatory variables in the model. In this analysis, brokenness and size were calculated relative to that of other group members. Relative values were used as the number of offspring produced may in part be determined by how effectively dominants can police subordinates, which may in turn depend on differences in RHP. However, absolute trait values may also be important if the number of offspring produced also relates to factors such as condition or fecundity. I therefore ran the analysis twice, using both relative and absolute values. Below I report the results for relative values of size and brokenness; substituting for absolute values does not alter the overall results.

4.3 Results

4.3.1 Population characteristics

Altogether, 3133 foundresses were marked in the period between nest-founding and worker emergence. The proportions of wasps with clypeal patterns at the three sites was: 13.4 % (Site 1), 21.5% (Site 2) and 26% (Site 3). The mean proportion of wasps with clypeal patterns across all sites was 20.3%. Morphological measurements were collected for 804 foundresses, of which 190 had clypeal patterns. These data were collected from 56 lone foundresses and 748 co-foundresses in 103 groups (in which ≥ 1 foundress had clypeal patterns). Across all 804 foundresses, mean pattern brokenness was 0.71 ± 0.11. Considering only wasps with clypeal patterns, mean brokenness was 2.86 ± 0.11 (range 0 - 8.28). Mean foundress size was 478.68 ± 1.20μm (range: 368.92 - 575.5μm). Size did not differ significantly between wasps with and without clypeal patterns (482.24 ± 2.19 vs. 477.69 ± 1.42μm; Mann-Whitney U test, $p = 0.17$). Furthermore, across all wasps, body size was not significantly correlated with brokenness (Spearman's $r = 0.05$, $p = 0.16$).

4.3.2 Lone foundresses reproductive success

A summary of the main results described here and in the following sections is presented in Table 4.1. Of the 56 lone foundresses for which productivity data were collected, 14 (25%) had clypeal patterns. Results of the analysis showed that foundresses with clypeal patterns did not have greater RS than those without patterns (Figure 4.2). Nests founded by wasps with clypeal patterns were no more likely to survive to the pupal stage than nests founded by wasps without patterns (ZINB model, $\chi^2_1 = 0.05$, $p = 0.83$). Furthermore, among nests that survived to the pupal stage, foundresses with clypeal patterns did not produce more pupae than foundresses without patterns ($\chi^2_1 = 0.07$, $p = 0.79$). In addition, there was also no significant effect of size on lone foundress success. Size did not predict survival to the pupal stage ($\chi^2_1 = 1.05$, $p = 0.31$) or the productivity of surviving nests ($\chi^2_1 = 1.66$, $p = 0.20$). Site also had no effect on lone foundress reproductive success ($\chi^2_2 = 0.64$, $p = 0.73$).

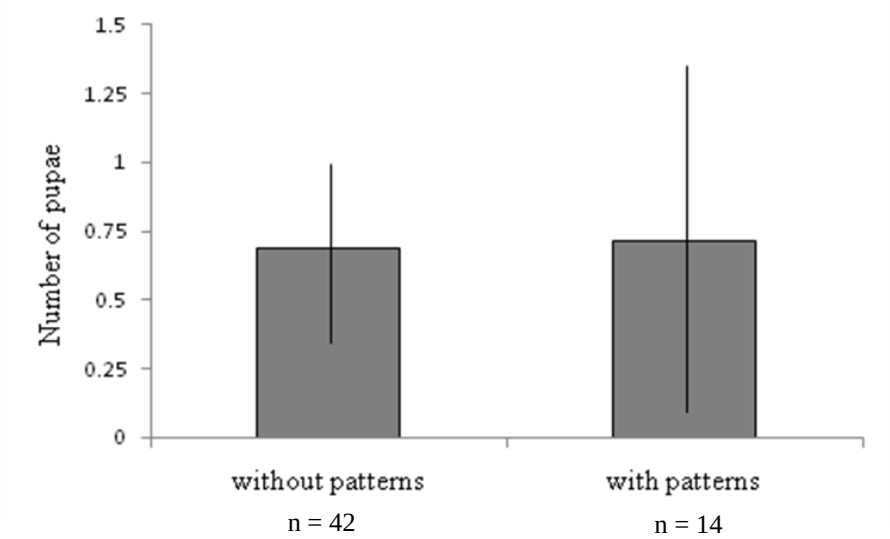


Figure 4.2 Numbers of pupae produced by lone foundresses with and without clypeal patterns. Means are shown ± 1 s.e.

4.3.3 Changes in group composition: survival and late-joining

The ratio of wasps with clypeal patterns to wasps without patterns did not change significantly over the two months from nest founding (Cochran's Q test, $\chi^2_2 = 0.85$, $p = 0.65$, $n = 146$ nests). Foundress survival in the month following nest stabilisation was not significantly predicted by brokenness ($\chi^2_1 = 0.65$, $p = 0.42$; Figure 4.3) or by size ($\chi^2_1 = 0.04$, $p = 0.85$), although the proportion of wasps surviving varied significantly between the two sites (77% vs. 51%, $\chi^2_1 = 10.32$, $p = 0.001$). To explore whether wasps with clypeal patterns were more likely to adopt a 'sit-and-wait' strategy, I compared the proportion of wasps with clypeal patterns that was marked prior to group stabilisation with the proportion of late-joiners with clypeal patterns arriving subsequently. There was no significant difference between the proportion of foundresses joining before group stabilisation with clypeal patterns and the proportion of late-joiners with clypeal patterns (19.58% vs. 20.73%; $\chi^2_1 = 0.08$, $p = 0.78$). Further, there was no difference in brokenness between early- and late-joining wasps ($Z = 0.18$, $p = 0.86$, $n = 170$ and 17 respectively). In a few cases, I was able to compare the clypeal patterning of early- and late-joiners in the same group. Within these groups, there was no difference in brokenness between early- and late-joiners ($Z = 0.84$, $n = 29$, $p = 0.46$).

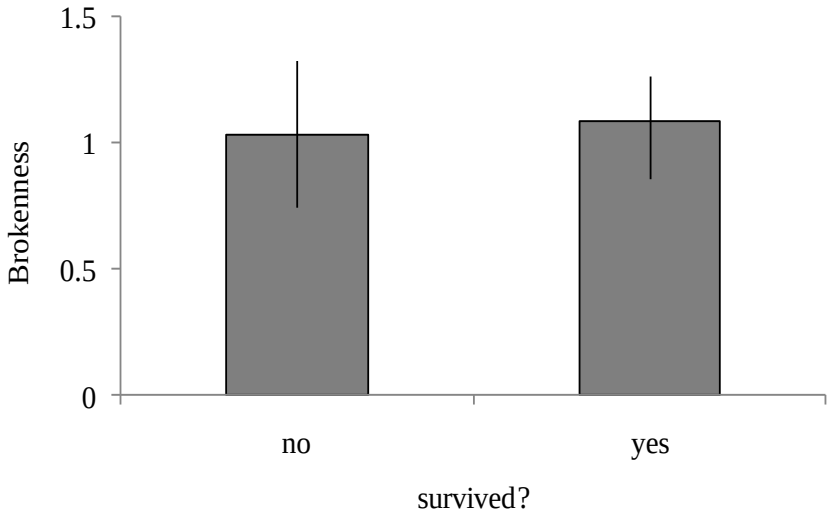


Figure 4.3 Brokenness vs. survival over a period of one month following group stabilisation. Means are shown ± 1 s.e.

4.3.4 Behavioural dominance in co-foundress groups

Results of the simulations showed that rank in the hierarchy was not significantly correlated with brokenness (mean ρ = -0.03, p = 0.42; Figure 4.4) or with size (mean ρ = -0.17, p = 0.65; Figure 4.5). Results of the GLM confirmed that rank was not predicted by a foundress' brokenness relative to her nest-mates (mean t = 0.74, $p < 0.05$ for 1.05% simulations). Similarly, the relative size of a co-foundress did not significantly predict her rank (mean t = 0.74, $p < 0.05$ for 1.85% simulations). Group size and site were also included in the GLM, but neither were found to predict rank (group size: mean t = 0.70, $p < 0.05$ for 1.25% simulations; site: mean t = 0.85, $p < 0.05$ for 4.45% simulations).

4.3.5 Reproductive success in co-foundress groups

Across all 38 co-foundress groups, 47 foundresses successfully produced one or more offspring; the remaining 205 foundresses produced no offspring. Results of the simulations showed that offspring number was not significantly correlated with brokenness (mean ρ = 0.03, p = 0.71; Figure 4.6a). Offspring number tended to be positively correlated with body size, though this was not statistically significant (mean ρ = 0.15, p = 0.08; Figure 4.7a).

Similar results were obtained when analysing co-foundress reproduction using GLMs. Whether or not co-foundresses produced offspring was not significantly predicted by brokenness (mean t = 0.94, $p < 0.05$ for 4.45% simulations; Figure 4.6b), and there was no significant interaction between brokenness and collection date (mean t = 0.90, $p < 0.05$ for 4.05% simulations). Similarly, size did not significantly predict whether or not a foundress produced offspring (mean t = 0.78, $p < 0.05$ for 3.25% simulations; size x collection date: mean t = 0.83, $p < 0.05$ for 4.2% simulations; Figure 4.7b). The likelihood of producing pupae was not significantly predicted by collection date (mean t = 0.88, $p < 0.05$ for 3.1% simulations) but was significantly negatively related to group size (mean t = 1.13, $p < 0.05$ for 11.4% simulations). The effect of group size is expected as larger groups contained more individuals that did not produce offspring, a consequence of the high skew in reproduction within co-foundress groups.

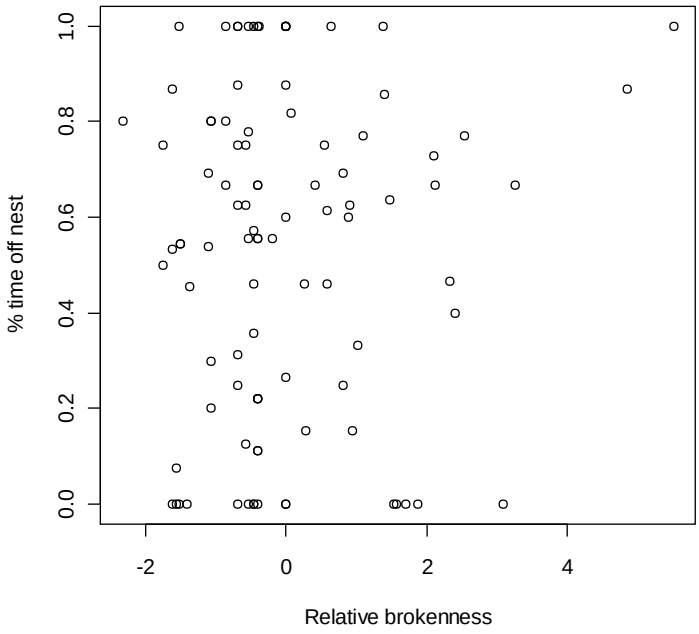


Figure 4.4 Relative brokenness vs. foraging effort (estimated as the proportion of time off the nest) for 97 foundresses in 18 co-foundress groups. Relative brokenness of each foundress in a group is calculated as its brokenness minus the average brokenness of all co-foundresses in that group.

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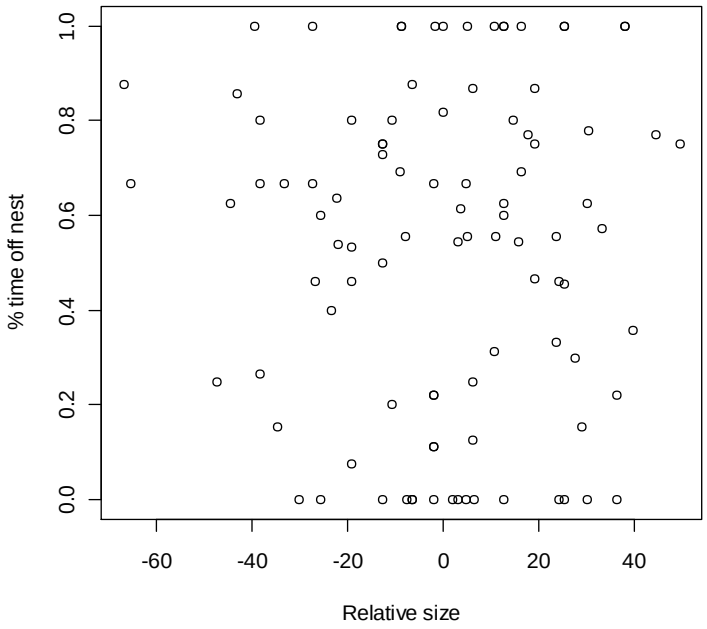

Figure 4.5 Relative size vs. foraging effort (estimated as the proportion of time off the nest) for 97 foundresses in 18 co-foundress groups. Relative size of each foundress in a group is calculated as its size minus the average size of all co-foundresses in that group.

Among the dominant reproductives in co-foundress groups, offspring number was not significantly related to brokenness ($F_{1,35} = 0.27$, $p = 0.61$; Figure 4.6c) and there was no significant interaction between collection date and brokenness ($F_{1,35} = 0.31$, $p = 0.58$). The analysis also showed that size did not have a significant effect on the number of offspring produced ($F_{1,35} = 0.05$, $p = 0.83$; size x collection date: $F_{1,35} = 0.29$, $p = 0.60$; Figure 4.7c). Collection date did significantly predict offspring number, with dominant reproductives on nests collected later in the season having more offspring than those on nests collected earlier in the season ($F_{1,36} = 5.35$, $p = 0.03$). However, there was no significant effect of either group size or site on offspring number (group size: $F_{1,35} = 1.67$, $p = 0.20$; site: $F_{2,34} = 1.32$, $p = 0.28$). Relative brokenness and size values were used in this analysis; the results remained the same when substituting for absolute values of brokenness and size.

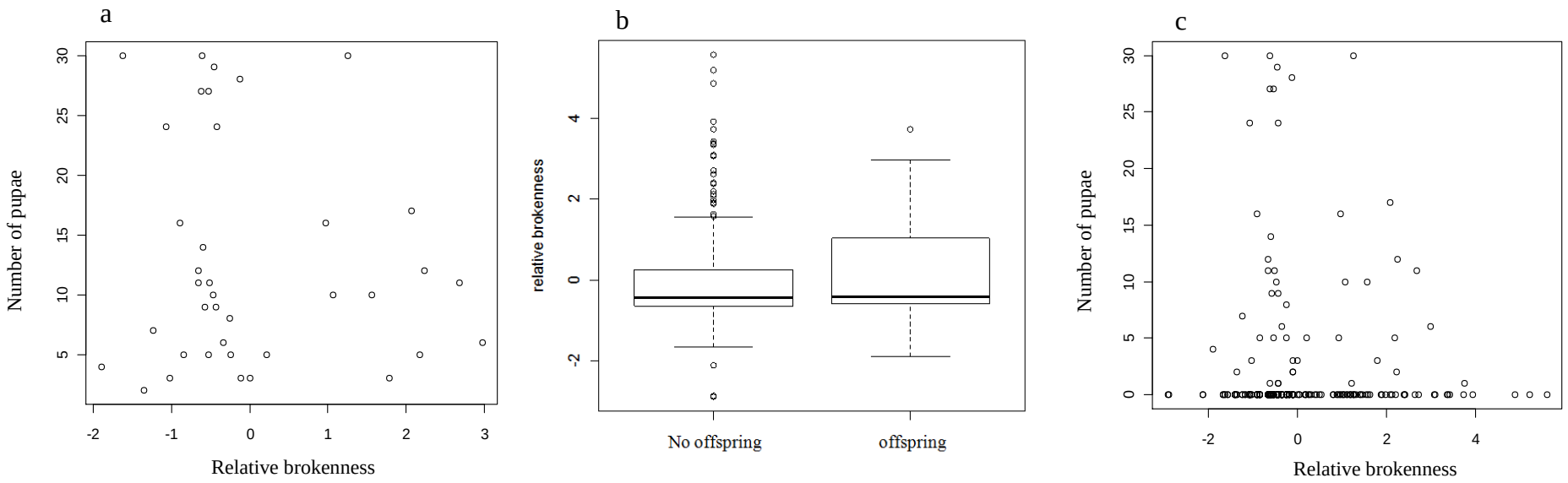

Figure 4.6. The relationship between pattern brokenness and RS for foundresses in 38 co-foundress groups. (a) Relative brokenness vs. number of offspring for all foundresses (*n* = 252). (b) Relative brokenness of co-foundresses that produced offspring and that did not produce offspring (*n* = 47 and 205 respectively). Thick black lines represent median values, the top and bottom lines of the box represent the first and third quartiles and vertical dashed lines represent approximately two standard deviations around the interquartile range (circles denote outliers). (c) Relative brokenness vs. number of offspring for dominant reproductives in each group. In all cases, relative brokenness of each foundress in a group is calculated as its brokenness minus the average brokenness of all co-foundresses in that group.

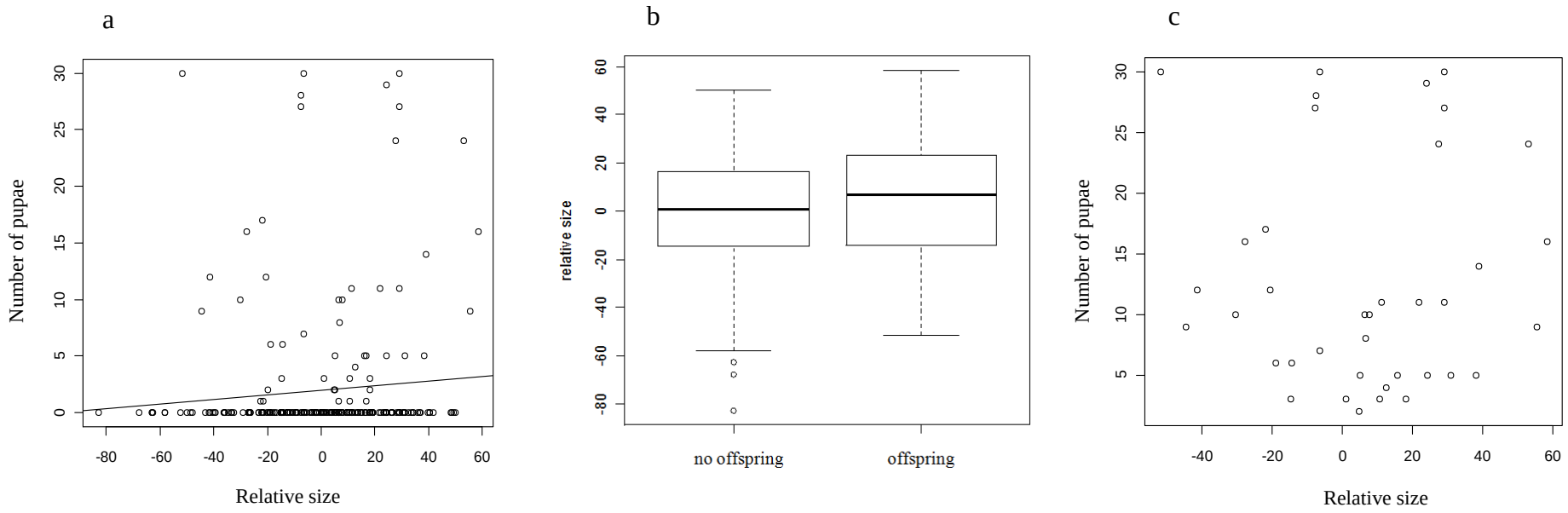

Figure 4.7. The relationship between body size and RS for foundresses in 38 co-foundress groups. (a) Relative size vs. number of offspring for all foundresses (*n* = 252). Least-squares regression line added for illustration. (b) Relative size of co-foundresses that produced offspring and that did not produce offspring (*n* = 47 and 205 respectively). Thick black lines represent median values, the top and bottom lines of the box represent the first and third quartiles and vertical dashed lines represent approximately two standard deviations around the interquartile range (circles denote outliers). (c) Relative size vs. number of offspring for dominant reproductives in each group. In all cases, relative size of each foundress in a group is calculated as its size minus the average size of all co-foundresses in that group.

Table 4.1. Summary of results from analyses exploring the influence of clypeal pattern brokenness and body size on various fitness measures in the wild. NS = non-significant effect or difference at significance cut-off of 0.05. *Statistical significance of variables in GLM analyses with random sampling determined following methods described in *Statistical Analyses* in sections 4.2.4 and 4.2.5.

Fitness measure	Brokenness	Size
Survival	NS effect	NS effect
Early *vs*. late nest joining	NS difference	Not explored
Behavioural dominance in co-foundress groups		
Behavioural rank	NS effect*	NS effect*
Behavioural dominants vs. subordinates	NS difference	NS difference
Reproductive success (RS)		
Lone foundress RS	NS effect	NS effect
Co-foundress RS: number of offspring (all wasps)	NS effect	NS positive correlation
Co-foundress RS: produced offspring or not?	NS difference*	NS difference*
Co-foundress RS: number of offspring (dominants)	NS effect	NS effect

4.4 Discussion

4.4.1 Brokenness and reproductive success

RS was estimated for both lone foundresses and co-foundresses. For lone foundresses, there was no relationship between the number of pupae produced and the brokenness of clypeal patterns. However, RS across all lone foundresses was very low, with the majority of lone foundresses failing to produce any pupae, which may have limited the power of the test to detect an effect of brokenness on offspring number. Lone-founding was also relatively rare across the three sites, with the majority of females nesting in groups. Whereas reproduction by lone foundresses is principally determined by survival and fecundity (Liebert *et al*. 2005), reproduction in co-foundress groups is thought to be determined in part by the competitive ability of group members (Röseler 1991). Given that brokenness is argued to be a signal of RHP, it might therefore be expected that brokenness more strongly predicts RS of co-foundresses than of lone foundresses. Surprisingly, however, this was not the case. There was no relationship between the number of offspring that a co-foundress produced and the brokenness of her clypeal pattern (this was the case whether considering absolute or relative values of brokenness). Likewise, no effect was found when distinguishing between females that produced pupae and those that did not, and when looking at the number of pupae produced by the dominant reproductive in the group. Furthermore, the absence of a significant interaction between brokenness and collection date in the GLM analyses indicates that brokenness had no significant impact on co-foundress RS at any point across the nesting season. Overall, then, there was no relationship between brokenness and RS, either for lone foundresses or co-foundresses.

While this result appears robust, there are a number of issues concerning the estimation and use of the RS data in this study that need to be addressed. First, RS was limited to counts of female offspring only. The exclusion of males from the analysis (necessitated by the high error rate in assigning males to foundresses) resulted in RS being underestimated for some co-foundresses. However, the exclusion of males would represent a problem only if male production was biased in favour of either more or less-broken individuals and there is no *a priori* reason to expect that this is so. Moreover, a

simple comparison of the proportions of foundresses to which males could be assigned with and without clypeal patterns shows that females with patterns are no more or less likely to produce males than those without ($\chi^2_1 = 1.71$, $p = 0.19$).

A second potential problem concerns the decision to estimate RS using offspring at the pupal stage of development. One issue with using pupae is that there is no information about offspring reproductive success, which is argued to be an important component of an individual's LRS (Hunt *et al*. 2004). However, the time in the season at which offspring are produced provides a good proxy for offspring success (at least for females), with mark-recapture experiments indicating that the majority of reproductive females are produced at the end of the season (Leadbeater *et al*. 2011). By including collection date in the analysis, I was able to test whether brokenness (or size) predicted RS at different times of the season, when offspring have different reproductive values. RS estimates based on pupal counts can be criticised for providing too little information on offspring success; however, they can also be criticised for incorporating too much information about offspring success (Clutton-Brock 1988). The impact of the environment, including the social environment, on offspring survival and condition is likely to be complex and highly variable, and may obscure the maternal contribution to offspring success (Clutton-Brock 1988). However, the alternative of estimating RS by genotyping eggs would likely be complicated by differential oophagy and egg replacement between co-foundresses (Gervet 1964; Liebig *et al*. 2005), such that reliable egg counts for each co-foundress would be difficult to obtain. Therefore, while not entirely problem-free, counts of pupae offered a practical and reasonably realistic estimate of foundress RS in this study.

4.4.2 Brokenness and hierarchical rank

The amount of reproduction that a foundress achieves at any given time is determined by her rank in the dominance hierarchy, with reproduction heavily skewed in favour of the dominant foundress (Queller *et al*. 2000; Leadbeater *et al*. 2011). It could therefore be argued that, by testing for correlations between both brokenness and reproduction and brokenness and hierarchical rank, I am merely performing the same test twice. However, even though subordinates collectively get very little reproduction, the rank

that a subordinate occupies is still important as it determines the likelihood of inheriting the dominant position, and thus gaining future reproductive benefits (Cant & Field 2001). Previous efforts to identify determinants of rank in *P. dominulus* have met with limited success (e.g. Zanette & Field 2009), though the intense fighting that occurs during group establishment has led several authors to suppose that asymmetries in RHP between co-foundresses are important in hierarchy formation (e.g. Reeve 1991; Röseler 1991). If this is the case, rank is expected to be positively correlated with brokenness, which is argued to reflect RHP. However, I found no correlation between brokenness and rank, indicating that brokenness does not reflect those aspects of quality that determine rank within co-foundress groups (including body size – see below).

4.4.3 Brokenness and survival

In vertebrates, breeding lifespan is often found to be the most important determinant of LRS (Clutton-Brock 1988). In *P. dominulus*, a female's lifespan across the nesting season will determine the number of offspring she produces where she is the dominant reproductive in a group or a lone foundress. For subordinates of a given rank, survival is positively correlated with the chance of inheritance of the dominant position. For all individuals, survival is likely to be affected by condition (e.g. health, nourishment), as well as by stochastic events (e.g. bad weather, nest predation). Foraging is also likely to impose survival costs, due to the depletion of energy stores and the increased risk of predation, and there is evidence that, among co-foundresses, the willingness to forage and incur these costs decreases as the likelihood of nest inheritance increases (Cant & Field 2001, 2005). In this study, I examined the relationship between brokenness and survival across a period of one month up to worker emergence. After the emergence of the first offspring, rates of disappearance of subordinates have been shown to increase, possibly as a result of increased conflict with the dominant (Hughes & Strassmann 1988; Reeve 1991; but see Savoyard & Gamboa 1999). By looking at survival before worker emergence, I therefore sought to minimise the risk of confusing disappearance due to death with disappearance due to eviction/voluntary departure. Although brokenness is primarily argued to reflect RHP, several studies have demonstrated a positive link between larval nourishment and brokenness (Tibbetts & Curtis 2007; Tibbetts 2010), suggesting that brokenness may also reflect aspects of individual

condition, and hence survival in the wild. However, this was not the case in my study: foundresses with more broken patterns did not enjoy greater survival over the pre-emergence period.

4.4.4 Brokenness and joining strategies

The final fitness measure that I explored was the joining behaviour of co-foundresses. The time in the season at which females join nests has been argued to reflect variation in individual quality (Starks 2001; Tibbetts *et al*. 2011b). In particular, a distinction has been made between individuals arriving before and after group stabilisation, with those arriving after deemed to be following a specific late-joining ('sit-and-wait') strategy (Starks 2001). Individuals may possibly adopt late-joining behaviours as a response to poor condition or reduced energy reserves, which prevent them from participating in nest founding, although there is little evidence to support this idea (Starks 2001). Rather, Starks (2001) has proposed that late-joiners seek to maximise direct fitness benefits by directing energy away from worker production and towards reproductive offspring later in the season. If late-joining is a strategy adopted by wasps in poor condition, fewer late-joiners may be expected to have broken patterns than early joiners. Conversely, if late-joining represents a strategy to secure reproduction on established nests, brokenness may be expected to be higher in general among late-joiners, and higher relative to established group members. Neither scenario was supported by the results: brokenness did not vary significantly between early and late-joiners, either within groups or across all groups. Clypeal pattern brokenness was therefore not associated with a potential late-joining strategy in this population.

4.4.5 Body size and foundress fitness

In a number of *Polistes* species, size has been shown to correlate with dominance (*P. dominulus*: Pardi 1948; Turillazzi & Pardi 1977; Cervo *et al*. 2008; *P. fuscatus*: Noonan 1981; *P. metricus*: Dropkin & Gamboa 1981). Many authors have therefore assumed that size is an important determinant of RHP (e.g. Röseler 1991), and that size asymmetries help to shape the dominance hierarchy. The limited data available on the role of size in fighting ability in *Polistes* broadly support this idea (Tibbetts & Shorter 2009; Ortolani & Cervo 2010; Cini *et al*. 2011; Green & Field 2011a; Chapter 6; but see

Cant *et al*. 2006). In this study, however, I found that size was not significantly correlated with hierarchical rank, which is consistent with previous research on co-foundress hierarchies in this population (Zanette & Field 2009). In Spain, therefore, hierarchies do not appear to be an outcome of size asymmetries between co-foundresses. However, in other *P. dominulus* populations in Italy, size and rank are positively correlated (Pardi 1948; Turillazzi & Pardi 1977; Cervo *et al*. 2008), indicating that population differences exist in the importance of size as a rank determinant, though the reason for such differences is currently unclear. Size was also found to be unrelated to foundress survival; however, the analysis did reveal a positive, though nonsignificant, correlation between size and offspring number. The observation that larger foundresses tended to produce more offspring suggests that large size might confer some kind of reproductive advantage within the Spanish population. For instance, while size does not appear to determine rank (nor, by extension, the identity of the dominant reproductive), it may be important in determining a dominant's productivity through an effect on condition or fecundity. A positive relationship between size and fecundity in particular is widespread among insects (Nylin & Gotthard 1998), and in *P. dominulus* may drive selection for large body size in females.

A major finding of this study was that body size and clypeal pattern brokenness were not positively correlated. In American populations, individuals with more broken clypeal patterns tend to be larger, which is cited as evidence that brokenness reflects RHP (Tibbetts & Dale 2004). Although significant, however, the correlation between size and brokenness reported by Tibbetts & Dale (2004) is very weak ($r^2 = 0.028$) and a second study by Tibbetts & Curtis (2007) found no association between brokenness and size. In Europe, there is no evidence for a positive correlation between brokenness and size (Cervo *et al*. 2008; this study). Thus, while size may be a determinant of RHP (see above), brokenness is neither a strong nor a consistent indicator of size in *P. dominulus*.

4.4.6 The quality basis of clypeal patterning

In *P. dominulus*, the brokenness of the clypeal pattern is argued to function as a signal of quality (Tibbetts & Dale 2004; Tibbetts 2006; Tibbetts & Curtis 2007; Tibbetts 2008; Tibbetts & Lindsay 2008; Tibbetts & Shorter 2009; Tibbetts 2010; Tibbetts & Banan

2010; Tibbetts & Izzo 2010; Tibbetts *et al*. 2010; Tibbetts *et al*. 2011a,b). This is based on three broad findings. First, brokenness predicts the outcome of contests staged in the lab (Tibbetts & Dale 2004; Tibbetts & Shorter 2009). Second, individuals react to pattern manipulations in a way that implies some kind of signal function (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010; Tibbetts & Izzo 2010). Third, brokenness is associated with aspects of individual condition, including nourishment and hormone titres (Tibbetts & Curtis 2007; Tibbetts 2010; Tibbetts *et al*. 2011a). Collectively, these findings have been used by Tibbetts and co-workers as evidence of a quality signal based on the brokenness of clypeal patterns. However, as compelling as these findings appear, they fall short of a clear and unequivocal demonstration that brokenness is an adaptation. As I argue in the Introduction, such a demonstration would require one to show that brokenness conveys a fitness advantage in the wild. However, in this study, the first to look for an association between brokenness and fitness, I was unable to detect a relationship between brokenness and any of the four fitness measures I investigated.

When exploring the relationship between a particular trait and fitness, Hunt *et al*. (2004) have emphasised the importance of looking across a range of quality components. Trade-offs between individual quality components can result in complex and varying relationships between individual components and total fitness, such that focusing on a single component may fail to give a true indication of the importance of a trait to individual fitness (Hunt *et al*. 2004). In this study I tested for associations between brokenness and a range of quality components, spanning survival, reproduction, individual condition and RHP. Given the existence of trade-offs between quality components, there is no strong reason to believe that brokenness, which is argued to signal RHP, ought necessarily to reflect other aspects of quality as well (cf. Cervo *et al*. 2008). The lack of an association between brokenness and survival, for instance, is therefore neither particularly surprising nor particularly damaging to the arguments in favour of brokenness as a status signal. What is surprising and potentially far more damaging, however, is the absence of any link between brokenness and rank or RS, both of which are thought to be strongly influenced by differences in RHP among group members. Indeed, Tibbetts and co-workers have proposed that status signals based on pattern brokenness should actually be of greatest importance during the period of

hierarchy establishment, signalling RHP to rivals during competition over rank and reproduction (e.g. Tibbetts & Lindsay 2008). Below, I explore alternative explanations for the finding that brokenness is not correlated with rank or RS, and in each case address the implications for the view of brokenness as an adaptation to competition.

The first explanation for the absence of a correlation between brokenness and rank and/or reproduction is that the analyses were not sufficiently powerful to detect an effect of brokenness. Given the numerous factors that are likely to impinge on an individual's survival and reproductive success, it is likely that the impact of any one factor will be fairly small, meaning that large sample sizes are required to detect an effect of a trait where it exists. The amount of variability in the trait is also likely to be an important limiting factor in such studies, with low trait variability reducing the power of tests to detect differences in fitness pay-offs between individuals with different phenotypes (Grafen 1988). In the Spanish populations, the proportion of wasps with black clypeal patterns (and therefore with a value of brokenness > 0) is only 15-20% (Zanette 2007; this study), meaning that there is no variation in brokenness among co-foundresses in the majority of co-foundress groups (i.e. all have an entirely yellow clypeus). Although I attempted to maximise the amount of variation in brokenness in the analysis by including only groups containing at least one individual with clypeal patterns, nonetheless the number of groups in each analysis was relatively small, which may have reduced the power of the tests to detect an effect of brokenness. One obvious solution to this problem would be to experimentally increase the amount of variation in brokenness by manipulating clypeal patterning in the field. Unfortunately, attempts to manipulate the clypeal patterns of free-living wasps (e.g. by using paint) have so far met with no success (J. P. Green, pers. obs.; E. A. Tibbetts, pers. comm.). Moreover, even if the pattern was a signal of status, changes to the pattern alone may be insufficient to produce changes in fitness in the presence of anti-cheating mechanisms that punish individuals whose clypeal pattern do not reliably reflect their underlying quality (Tibbetts & Dale 2004; Tibbetts & Izzo 2010; Chapters 1 & 3).

Alternative explanations for the results assume that that the absence of an effect of brokenness on fitness in the Spanish population is not a result of low statistical power but is a real phenomenon. In the previous chapter, I presented evidence that brokenness

does not have any signal value during contests in the Spanish population. The results presented here extend that finding considerably by showing that brokenness does not predict quality (including RHP) among foundresses in the wild. In America, data from experimental studies purport to demonstrate status signalling via brokenness. However, certain features of these studies, including elements of experimental design and analysis, call for caution when interpreting them (see Chapter 1). More importantly, as I have argued above, in the American populations we lack a detailed analysis of the relationship between brokenness and fitness of the kind presented here. Without this, it is not possible to determine unequivocally whether brokenness in these populations is truly an adaptation. If brokenness is found to have adaptive value in American populations, this would point to a significant divergence in signalling between American and Spanish populations. Variation in the use of sexually-selected traits between populations has been recorded in a number of species (e.g. Wilczynski & Ryan 1999; Dunn *et al*. 2008; Takahashi *et al*. 2008). Differences in environmental conditions between populations are one factor known to be important in maintaining intraspecific variation in the form and function of sexually-selected traits (Wilczynski & Ryan 1999). In the following chapter, I test the hypothesis that environmental conditions (specifically, climatic conditions) are responsible for generating variation in signalling between different populations of *P. dominulus* through climate-mediated selection on correlated traits.

An alternative, and more parsimonious explanation for the results presented in this study exists, however. This is that brokenness may simply not have any adaptive value in the wild, either in Spain or in North America. To date, the majority of studies seeking to demonstrate an adaptive function for the clypeal patterns have been based on experiments in the laboratory. Some of these studies have involved staged contests between wasps in the absence of a fitness-enhancing resource over which they can compete (Tibbetts & Dale 2004; Tibbetts & Izzo 2010; Tibbetts *et al*. 2011a), while others have attempted to demonstrate signalling by testing receiver responses to manipulations performed on dead individuals (Tibbetts 2008; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). While such designs are useful for testing receiver responses to signal manipulations, they do not provide any information on the functional significance of the patterns in the wild. In contrast, only two studies have attempted to explore

brokenness in the wild: Tibbetts (2007) found that workers disappearing from the nest early in the season did not have more broken patterns than those remaining on the nest, while Tibbetts (2006) found that offspring emerging later in the season had more broken patterns than those emerging earlier in the season. In the case of Tibbetts (2007), however, it is not apparent what a significant difference in brokenness between the two groups of workers, if found, would have indicated, while the finding of Tibbetts (2006) does not obviously demonstrate any link between brokenness and fitness in the wild.

The history of research into the adaptive significance of the variable clypeal pattern in *P. dominulus* appears strikingly back-to-front. Complex hypotheses for honesty-regulating mechanisms are routinely tested in an attempt to solve the paradoxes of status signalling (Tibbetts & Dale 2004; Tibbetts & Izzo 2010), while other studies have set about exploring the physiological and hormonal basis of individual quality, as advertised by pattern brokenness (Tibbetts & Izzo 2009; Tibbetts & Banan 2010). Yet, astonishingly, for almost a decade this activity has flourished in the absence of any evidence that clypeal patterning is in any way related to fitness in the wild. The results of my study provide strong evidence that, in a European population, clypeal patterns have no adaptive value in the wild. If we are to better understand this result, and its implications for status signalling in *P. dominulus* in general, an investigation into how brokenness relates to fitness in American populations must now be a priority.

4.5 Summary

In Chapter 3, I showed that clypeal patterns do not signal status in Spanish populations of *P. dominulus*. In this chapter, I examined the relationship between clypeal patterning and quality in *P. dominulus*. I found no evidence that pattern brokenness reflects individual quality in the wild. A potential explanation for these findings is that there is variation in the use of the clypeal pattern as a status signal between populations, potentially as a result of differing environmental conditions. In the following chapter (Chapter 5), I explore the factors responsible for variation in clypeal pattern expression between populations. Using a simple brood rearing experiment, I show that expression of the clypeal pattern is influenced by the thermal environment during development.

Chapter 5: Climate-driven variation in a status signal in *Polistes dominulus*

5.1 Introduction

5.1.1 Intraspecific variation in sexual signalling

Within a species, the signal value of a trait under sexual selection is not always constant but can vary from population to population (Endler & Houde 1995; Griffith *et al*. 1999b; Dunn *et al*. 2008; Green & Field 2011b; Chapter 3). Geographic variation of this kind appears at odds with the process of sexual selection, which acts to reduce variation in traits that affect reproductive success within species (Bussière *et al*. 2008). Recent research has therefore focused on identifying the factors that influence inter-population variation in sexually-selected traits as a step towards understanding how additive genetic variation in both signals and receiver responses can be maintained in the face of sexual selection (Hill 1994; Griffith *et al*. 1999b). One factor known to be important in maintaining variation in sexual signals within a species is variation in the environmental conditions experienced by different populations, which can drive changes in signal form and information content, as well as in receiver responses (e.g. Endler & Houde 1995; Griffith *et al*. 1999b; Roulin *et al*. 2008; for a review see Wilczynski & Ryan 1999).

Divergence in signal form may be driven by differences in the efficacy of signal transmission between habitats (Endler & Houde 1995; Gamble *et al*. 2003) or by natural selection for greater crypsis in habitats where predators are at high density (Endler 1980; Zuk *et al*. 2006). For example, in guppies (*Poecilia reticulata*), the specific elements of male coloration used in mate choice by females vary with water colour and ambient light (Endler & Houde 1995; Gamble *et al*. 2003) and with the risk of detection by visual predators (Endler 1980). Divergent selection on traits correlated with the signalling trait can also drive variation in signal form: in cases where the strength and/or direction of selection on correlated traits varies across environments, the form of the signal, as well as the underlying variation in the signalling trait, may therefore also vary (Wilczynski & Ryan 1999). For example, in frogs, body size is important for determining how calls of particular frequencies are produced by males and perceived by

females. Divergent selection on body size in other contexts (e.g. as an adaptation to local climatic conditions) may therefore drive variation in male calls and female responses between populations (Wilczynski & Ryan 1999, and references therein).

The information that a sexual signal provides about the genetic quality of its bearer can also be sensitive to variation in the environment. In particular, there is evidence from studies of birds for the effects of genotype-by-environment interactions (G×E) on intersexual signalling (Qvarnström 1999; Roulin *et al*. 2008). G×E has been shown to maintain variation in secondary sexual traits via differences in the relative fitness of genotypes across environments. In birds, for instance, melanin-based coloration often functions in sexual signalling and in Alpine swifts (*Apus melba*) is associated with rapid offspring growth in poor environments but not in rich environments (Roulin *et al*. 2008). Furthermore, there is evidence that G×E effects on sexual signals can promote divergence in receiver responses to signals between environments. Evidence from mate choice studies in house sparrows indicates that the success of males signalling at a given intensity varies with environment, and that this has driven variation in female preferences among populations. In environments where nest-sites are scarce, females prefer to mate with males with large bibs as these males are more aggressive and thus more successful in acquiring territories. However, in environments where nest-sites are plentiful, females are observed to prefer males with small bibs as these males are less aggressive and devote relatively more time to chick provisioning (Griffith *et al*. 1999b).

To date, evidence of intraspecific variation in sexual signalling comes largely from studies of intersexual signals i.e. those involved in mate choice (e.g. Endler & Houde 1995; Griffith *et al*. 1999b; Wilczynski & Ryan 1999). In contrast, very little is known about the scope for inter-population variation in the signal value of intrasexually-selected traits (but see Baird *et al*. 1997; Wong *et al*. 2007). Intrasexual signals are widespread in nature and are important in mediating competition within sexes for access to resources that enhance reproductive success (Maynard Smith & Harper 2003). Like intersexual signals, intrasexual signals also show condition-dependence (Maynard Smith & Harper 2003), and studies have shown that their development is sensitive to environmental conditions (Griffith *et al*. 1999a; Tibbetts & Curtis 2007). Intrasexual

signalling may therefore also show variation based on differences in environmental conditions between populations (e.g. Baird *et al*. 1997), though this has yet to be tested.

5.1.2 Intraspecific variation in status signalling in *Polistes dominulus*

In this study, I explore factors responsible for inter-population variation in intrasexual signalling in the paper wasp, *P. dominulus*. *P. dominulus* is a temperate species with a widespread distribution, having undergone several major expansions in recent years from its native range in Africa and Eurasia into North America, South America and Australia (Liebert *et al*. 2006). In North America, experimental evidence exists for the use of an intrasexual signal (status signal) during competitive interactions between females (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). The basis of the signal is melanin patterning on the clypeus, which is fixed throughout adult life and varies among wasps from no black patterns (i.e. a completely yellow clypeus) to black patterns of variable size and shape (Tibbetts & Dale 2004; Chapter 1). Previous work suggests that an individual's agonistic ability is signalled by the degree of disruption (brokenness) in the pattern: individuals with wavy or spotty patterns signal a greater competitive ability than those with more uniform patterns or no black patterns (yellow clypeus) (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008). Thus, variation in brokenness within a population depends partly on variability in the amount of black on the clypeus among wasps.

Within a single population, the amount of black on the clypeus is heritable, and unlike brokenness, varies little with factors such as larval nourishment (Tibbetts 2010), although there is variation in the amount of black between females emerging early in the season (workers) and those emerging later in the season (reproductives) (Tibbetts 2006). Across populations and geographical regions, however, variation in the amount of black on the clypeus is striking. In North America, variability in the amount of black is high: around 80% of wasps have black patterns of varying size and shape. In Mediterranean populations, however, variability in the amount of black is much lower, with as many as 80% of wasps with a completely yellow clypeus. Moreover, population differences in the variability of clypeal patterns appear to be associated with variation in the signal value of clypeal patterns between populations. In North America, where pattern

variability is high, clypeal patterns are used to signal fighting ability to rivals; in Spain, however, where pattern variability is low, patterns have no signal value during competition (Green & Field 2011b; Chapter 3) and do not appear to reflect individual RHP in the wild (Chapter 4).

Here, I explore whether variation in environmental conditions, specifically variation in climatic factors, is responsible for the differences in the clypeal patterning between populations. In insects, the influence of climatic factors such as temperature and humidity on the development of melanin-based patterns is well-documented (Hintze-Podufal 1977; Tsuruta *et al*. 1989; for a review see Nijhout 1999). Among wild populations, polymorphism in the degree of melanisation often reflects local adaptation to temperature, with melanic forms more common at higher latitudes and altitudes where they show enhanced thermoregulatory ability compared to lighter forms (reviewed in True 2003). In *P. dominulus*, temperature may maintain variation in clypeal patterning across populations. In support of this idea, data on climatic conditions and clypeal patterning from five populations show a negative trend between the proportion of wasps with clypeal patterns and ambient temperature during brood development (Figure 5.1). Humidity is also thought to play a role in maintaining variation in melanisation within species. For example, populations of the fruit fly *Drosophila melanogaster* living at high altitude exhibit greater body melanisation, which has been shown to protect against desiccation (Parkash *et al*. 2008). Climate-dependent expression of clypeal patterning (via direct selection on patterning, indirect selection on correlated traits or G×E) may account for the differences in pattern variability between populations and, in doing so, may also explain the variation in the signal value of clypeal patterns between populations.

To test for effects of climatic factors on the expression of clypeal patterns in *P. dominulus*, I return to the pioneering work of Enteman (1904) on *Polistes* coloration. Experiments in which pupae of *P. fuscatus* (then referred to as *P. variatus*) from a nest were reared in different temperature and humidity regimes found effects of both factors on the extent and degree of melanisation of the eclosing adults (Enteman 1904). To test for climatic effects on clypeal patterning in *P. dominulus*, I compared the amount of black on the clypeus of wasps reared under different temperature and humidity regimes.

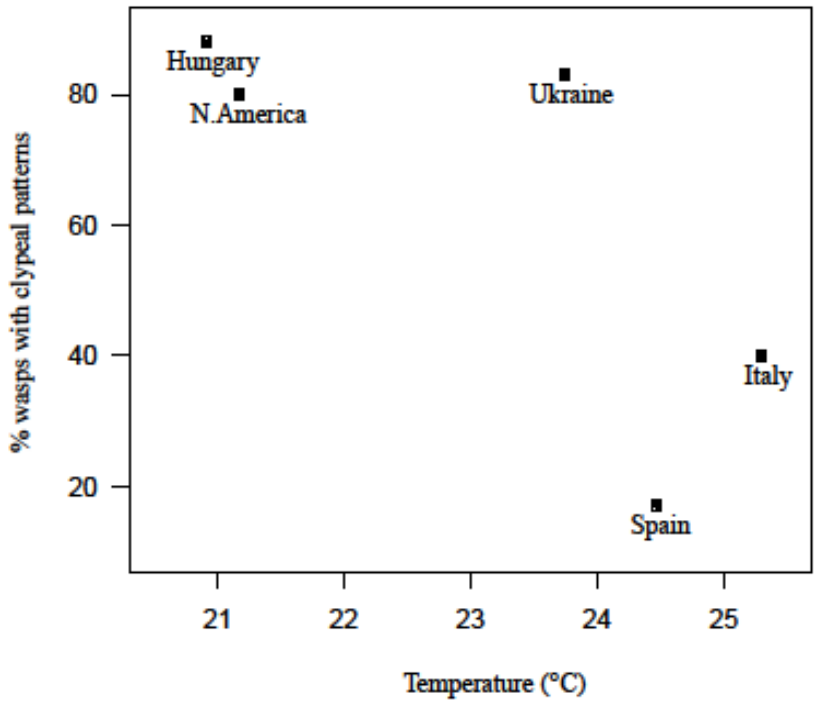

Figure 5.1 Prevalence of clypeal patterns in relation to ambient temperature (°C) during the period of late worker and gyne development across five geographical areas*. Data represent mean daytime temperatures for June-July (Spain) and July-August (Hungary, Italy, N. America, Ukraine) over a 10-year period (2001-2010) †.

*Clypeal pattern and colony cycle data from: Hungary (J. P. Green & Z. Ács, unpubl. data); Italy (Queller *et al*. 2000, Cervo *et al*. 2008); N. America (Tibbetts & Dale 2004, Tibbetts 2006); Spain (Zanette 2007, Leadbeater *et al*. 2011); Ukraine (Rusina *et al*. 2006).

†Temperature data obtained from the following weather stations: Florence 161700 (Italy), Ithaca, NY 725155 (N. America), Jerez 84510 (Spain), Kherson 339020 (Ukraine) & Veszprem 128300 (Hungary). Data accessed at www.tutiempo.net/en/.

5.2 Materials and Methods

5.2.1 Rearing Experiment

In early June 2010, I collected 20 large *P. dominulus* nests from farmland near Conil de la Frontera (Cádiz Province, Spain) during the reproductive phase of the colony cycle. At this stage, the majority of females that emerge are reproductives (Leadbeater *et al*. 2011, online supplementary material), and will over-winter to found nests in the following spring. The rearing experiments were carried out at the University of Sussex, UK. Nests were divided into four pieces, each piece of roughly equal size and containing a variable number of pupae (>4, mean ± 1 s.d. = 16.2 ± 7.8). Nest quarters were placed individually in small plastic pots, with wire mesh over the top, and housed in identical SANYO growth chambers (MR350 model; SANYO E&E Europe BV, Loughborough, UK). In some insect species, the development of melanin patterning is sensitive to light/photoperiod (Nijhout 1999). In order to control for any effects of light on clypeal pattern development, nests were kept in constant darkness. Each nest quarter was reared under one of the following four temperature-humidity regimes: 20°C/40% RH, 20°C/65% RH, 30°C/40% RH and 30°C/65% RH. The levels of humidity and temperature used were within the natural range experienced by wasps in the wild. I collected all wasps emerging from the nests over the course of the experiment. However, I only took morphological measurements from females that eclosed 7 days after the start of the experiment (females eclosing before this time were not included in the analysis as they had been exposed to the experimental conditions for only a short period of their development). Thereafter, I collected newly-eclosed females at regular intervals (every two days) for three weeks, after which point no more wasps emerged. Males were not collected for analysis as they do not have variable clypeal patterns. Parasitoids (*Latibulus argiolus*) emerging on 18 nests were also discarded.

5.2.2 Morphological measurements

After collection, wasps were killed by freezing. The head of each wasp was removed using a scalpel and mounted, and a photograph of the clypeus was taken using a Nikon D80 digital camera (with macro lens) from a fixed distance and under constant lighting conditions. Images analysis was performed to calculate the proportion of the clypeus

pigmented black and the 'brokenness' index of the pattern (see Chapter 2). Body weight was recorded as the dry weight of each wasp (minus the head). Wasps were weighed following drying in an oven at 60°C for 8 days using a balance accurate to 0.0001g.

5.2.3 Statistical Analyses

Previous work by Tibbetts (2010) has demonstrated a heritable component in the amount of black laid down on the clypeus. This finding indicates that clypeal patterns are likely to be more similar among females emerging on the same nest (who are often sisters) than between females emerging on different nests. Results of a GLM with binomial errors in which presence/absence of the clypeal pattern was fitted as the response variable and nest as the explanatory variable confirmed that there was significant between-nest variation in clypeal patterning ($\chi^2_{19} = 67.8$, $p < 0.0001$). To control for differences in clypeal patterning between nests, I therefore used mixed-effects models in all the analyses, where 'nest' was fitted as a random factor.

To test the effect of climate on the presence of black clypeal patterns, I used a GLMM with binomial errors where presence/absence of clypeal patterns was fitted as the binary response variable. Temperature, humidity and body weight were fitted as explanatory variables. The interaction between temperature and humidity was also fitted as an explanatory variable to test whether the effect of one climatic factor was dependent on the level of the other. To test the effect of climate on pattern brokenness, I used a LMM where brokenness (log-transformed) was fitted as the response variable. As before, temperature, humidity, body weight and the interaction between temperature and humidity were fitted as explanatory variables. Means are reported ± 1 s.e.

5.3 Results

5.3.1 Presence of black patterning

Morphological data were collected for 223 individuals eclosing within the collection period. Thirty-five wasps eclosed at 20°C/40% RH, 29 at 20°C/65% RH, 87 at

30°C/40% RH and 72 at 30°C/65% RH. There was a highly significant effect of temperature on the amount of black on the clypeus: wasps reared at 20°C were 68% more likely to have black patterns than those reared at 30°C ($\chi^2_1 = 16.71$, $p < 0.0001$; Figure 5.2). However, the presence of black patterns was not influenced by humidity ($\chi^2_1 = 0.34$, $p = 0.56$). Furthermore, the interaction between temperature and humidity was not significant ($\chi^2_2 = 0.71$, $p = 0.70$), indicating that the effect of temperature on clypeal patterns was not dependent on humidity. The analysis also showed no effect of body weight on the presence of clypeal patterns ($\chi^2_1 = 2.14$, $p = 0.14$).

5.3.2 Pattern brokenness

Developmental temperature was also found to affect the brokenness of the clypeal pattern. Wasps eclosing at 20°C had significantly higher brokenness than wasps eclosing at 30°C ($L_1 = 9.49$, $p = 0.002$; Figure 5.3). However, there was no effect of humidity on brokenness, either directly ($L_1 = 2.01$, $p = 0.16$) or via an interaction with temperature ($L_2 = 2.10$, $p = 0.35$). There was also no significant relationship between body weight and brokenness ($L_1 = 0.84$, $p = 0.36$).

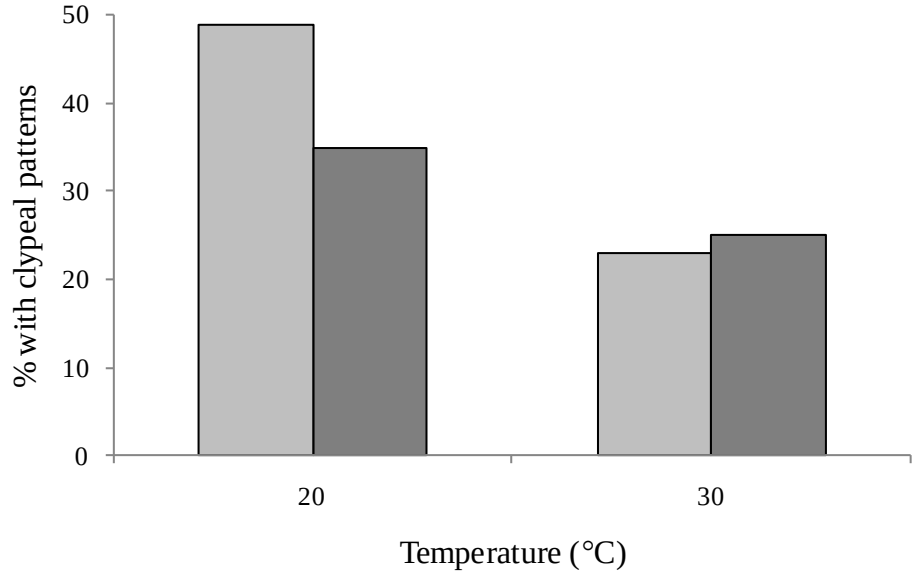

Figure 5.2 Proportion of wasps with clypeal patterns eclosing under each of the temperature-humidity regimes. Light grey bars represent 40% humidity and dark grey bars represent 65% humidity.

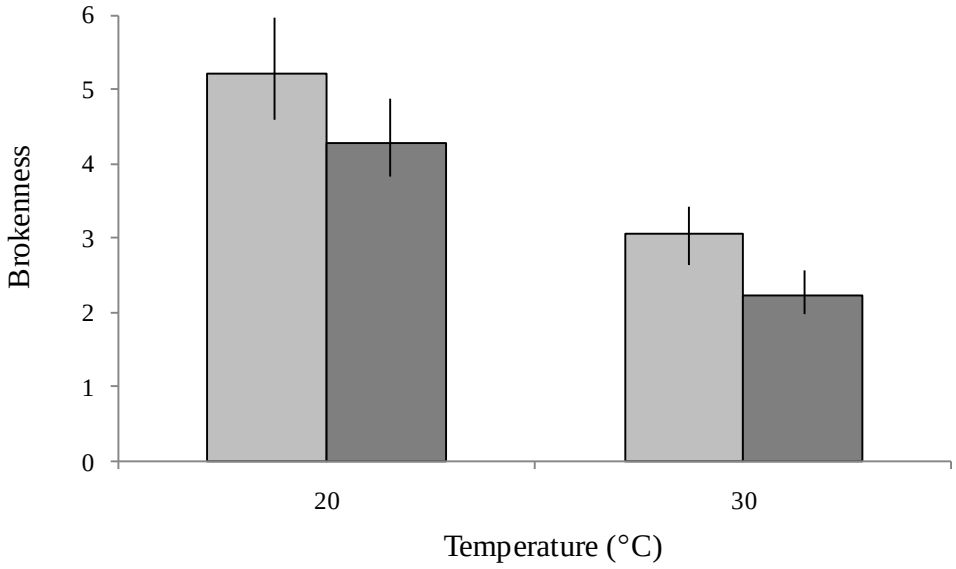

Figure 5.3 Mean brokenness (untransformed) for wasps with clypeal patterns under each of the temperature-humidity regimes. Light grey bars represent 40% humidity and dark grey bars represent 65% humidity. Means are shown ±1 s.e.

5.3.3 Developmental plasticity vs. differential survival

The emergence of more patterned wasps (and wasps with more broken patterns) at 20°C than at 30°C supports the idea that temperature experienced during development affects the amount and arrangement of melanin on the clypeus. However, this finding could also be explained by differential survival of wasps with and without patterns at 20°C. Clypeal patterns are argued to reflect aspects of individual quality, with higher-quality individuals developing more broken patterns (Tibbetts & Curtis 2007; but see Chapter 4). In this study, temperature had a pronounced effect on pupal development, with pupae reared at 20°C eclosing on average 8 days later than those reared at 30°C (18 ± 0.77 days vs. 9.58 ± 0.19 days). Results of a GLMM with poisson errors with eclosion date as the response variable and temperature, humidity and weight was explanatory variables confirmed that this effect was highly significant ($\chi^2_1 = 231.00$, $p < 0.0001$). Lower temperatures may therefore have resulted in a reduction in survival among pupae via an increase in pupal development time. The higher brokenness of individuals at 20°C could therefore be a consequence of a survival bottleneck during development, biasing survival towards high-quality individuals with clypeal patterns.

The differential survival hypothesis makes two testable predictions. First, if lower temperatures impose a bottleneck on survival, then overall survival is expected to be lower at 20°C than at 30°C. However, the results of a GLMM with binomial errors revealed no significant difference in the proportion of pupae successfully eclosing and those failing to eclose (here including males and wasps eclosing in the first 7 days of the experiment) between 20°C and 30°C (47% vs. 52% survival; $\chi^2_1 = 2.81$, $p = 0.09$). Second, if among wasps eclosing at 20°C those with patterns are of higher quality, then they might be expected to differ in other characters linked with quality such as body weight (Tibbetts 2006). However, a LMM revealed no significant differences in weight between wasps with and without clypeal patterns (22 ± 0.6 vs. 20 ± 0.5 mg; $L_1 = 0.88$, $p = 0.35$). Taken together, these results are more consistent with temperature-driven changes in the development of clypeal patterns than with differential survival of wasps with and without patterns.

5.4 Discussion

Identifying the causes of variation in sexually-selected traits is crucial to understanding how such variation can be preserved in the face of sexual selection. The results of this study show that expression of an intrasexually-selected trait whose signal value varies between populations is dependent on thermal conditions during development. Wasps from the same natal nest reared in cooler conditions were more likely to have black clypeal patterns than those reared in warmer conditions. Furthermore, wasps laying down melanin produced more broken clypeal patterns in cooler conditions than in warmer conditions. Importantly, the effect of temperature was significant when controlling for similarities between wasps eclosing on the same nest. Furthermore, since the majority of individuals eclosing in the collection period were reproductives, differences in clypeal patterning between workers and reproductives reported elsewhere (Tibbetts 2006) are unlikely to account for this result. However, in contrast to the strong effect of temperature on clypeal patterning, there was no significant effect of humidity either on the probability of laying down melanin patterns, or on the specific arrangement of melanin on the clypeus.

In several studies, clypeal patterning has been shown to correlate positively with body size (e.g. Tibbetts & Dale 2004). Body weight was therefore included as a covariate in the analyses to control for an association between patterning and size. As in the previous chapter, however, I found that body weight predicted neither the likelihood of having clypeal patterns, nor the brokenness of these patterns. Tibbetts & Curtis (2007) have previously argued that manipulation of environmental factors during development (in that study, larval food supply) can obscure the positive association between patterning and body size. While temperature-induced stress during development could possibly account for the absence of a positive correlation between patterning and size (weight) in this study, a similar result was also obtained in the previous chapter (Chapter 4). There, measurements of body size and clypeal patterning were taken from wild-caught foundresses from the same Spanish population (albeit in a different year). Together, therefore, these findings suggest that the absence of a relationship between size and patterning is not an artefact of the rearing experiment but rather a genuine feature of the Spanish population. The extent to which clypeal patterns truly reflect body size within different populations has important implications for their use as RHP signals in this species (for further discussion see Chapters 4 and 8).

The main finding of this study was that clypeal patterning varied with temperature during development. However, the emergence of a greater proportion of wasps with clypeal patterns at 20°C does not necessarily demonstrate an influence of temperature on pattern development; a similar result might be expected if wasps with patterns are better able to withstand the lower temperatures (and/or the associated increase in development time) than those without patterns. Two further findings make this explanation unlikely, however. First, brood survival did not differ significantly between temperature regimes, indicating that conditions at 20°C did not act as a bottleneck biasing survival towards patterned wasps. Second, patterned wasps emerging at 20°C were not larger than wasps without patterns, which might be expected if patterned wasps were of higher quality and enjoyed greater survival at lower developmental temperatures. There is thus little evidence for greater survival and/or performance of patterned wasps in cooler conditions. This suggests that the difference in patterning between temperature conditions is unlikely to be the result of a phenotype-by-

environment interaction but rather is consistent with phenotypic plasticity in the development of the clypeal pattern in response to temperature.

5.4.1 Temperature-driven variation in clypeal patterning

As noted above (see Introduction), there are remarkable differences between populations of *P. dominulus* in the amount of black on the clypeus, with wasps inhabiting cooler environments more likely to have black patterns (Figure 5.1). The result of this study indicates that the correlation between patterning and temperature is not incidental, but that differences in the thermal environment during development could in fact account for the variation in patterning that we observe in the wild. Indeed, the negative trend seen across populations between the proportion of wasps with black patterns and temperature mirrors that observed in other insect species where melanin patterns are known to play a role in thermoregulation (reviewed in True 2003). In these species, individuals living at high altitudes or latitudes possess a greater amount of black on the body cuticle as this facilitates more rapid heat absorption under cold conditions (True 2003). Furthermore, in many of these species, melanisation of the cuticle exhibits plasticity in response to environmental cues, including temperature or associated cues such as development time (e.g. Goulson 1994; Marriott & Holloway 1998). Thus, selection for efficient thermoregulation in different climates may drive variation in melanin-based patterning across different populations of *P. dominulus*, although it remains to be shown whether wasps with clypeal patterns have more black on the body as a whole, thereby allowing for greater heat absorption in cooler habitats.

Moreover, the finding that temperature affects clypeal pattern expression provides a potential explanation for the variation in the use of status signals across populations. The observation that signalling via clypeal patterns is restricted to populations where pattern variability is high (i.e. where many wasps have black patterns of varying shape and size) suggests that the amount of variation in patterning may constrain signal function. Although brokenness and the amount of black are two distinct measures of the clypeal pattern, variation in the former is dependent upon variation in the latter: where very few wasps have black patterns, there is little scope for significant variation in brokenness in the population. Environmental factors favouring reduced amounts of

black (e.g. high temperatures) in some populations may therefore effectively preclude status signalling via pattern brokenness. In Spain, high temperatures selecting for reduced clypeal patterning could account for the absence of signalling in this population. Conversely, in northeastern North America, cooler temperatures during offspring development could facilitate signal use via an increase in pattern variability among wasps. Previous studies have shown both that environmental factors can constrain variation in components of sexual signals (e.g. Grether *et al*. 1999) and that the requirement for thermoregulation in particular can constrain selection on colour-based signalling (Lindstedt *et al*. 2009). In *P. dominulus*, climate-driven variation in the amount of black laid down on the clypeus, potentially as a result of selection for efficient thermoregulation, may be responsible for driving variation in signal value of patterns between populations. A good test of this hypothesis would be to compare the signal value of clypeal patterns between other locations where clypeal pattern variability differed, for example in Italy and Ukraine, where pattern variability is low (Cervo *et al*. 2008) and high (Rusina *et al*. 2006) respectively.

5.4.2 Variation in the information content of clypeal patterns

Previous studies of sexual signalling have proposed that, in addition to driving variation in signal form (that is, in the structure and variability of signalling traits), environmental variation can also affect the type of information conveyed by signals (e.g. Griffith *et al*. 1999; Roulin *et al*. 2008), which in turn can affect how receivers respond to these signals (Griffith *et al*. 1999b). In this study, wasps reared at cooler temperatures were not only more likely to develop clypeal patterns, but in addition developed more broken clypeal patterns. In North America, pattern brokenness has been argued to reflect aspects of individual quality. Experimental manipulations of larval food supply have shown that brokenness is sensitive to larval nourishment, with better-fed wasps developing more broken patterns that signal their superior condition (Tibbetts & Curtis 2007; Tibbetts 2010). A similar explanation has been given for the variation in clypeal patterning observed among offspring in a wild North American population, where more broken patterns in late-emerging offspring is argued to be the result of increased food availability later in the season (Tibbetts 2006). However, while brokenness is thought to reflect quality in North America, in Europe there is little evidence that brokenness

reflects individual quality across a wide range of contexts, including health, survival, dominance and reproductive success (Cervo *et al*. 2008; Chapter 4). Thus, pattern brokenness appears to provide different information about individual quality across different populations.

In light of these findings, how can we account for the effect of temperature on pattern brokenness found in this study? One explanation may be that the arrangement of melanin on the clypeus provides information about some aspect of phenotype that is sensitive to temperature during development. For instance, higher brokenness at 20°C may be the result of increased synthesis of (or sensitivity to) juvenile hormone (JH). In several insect species, environmentally-induced variation in JH titres is responsible for variation in melanisation between individuals (Nijhout 1999). In *P. dominulus*, JH titres are positively correlated with both brokenness and RHP (Tibbetts *et al*. 2011a), and it has been suggested that effects of JH on pattern development may provide the basis for honest signalling of RHP via pattern brokenness (Tibbetts & Banan 2010; Chapters 1 and 8). An increase in JH as a result of exposure to low temperatures may therefore result in the more broken patterns seen in wasps eclosing at 20°C.

There are several potential problems with this explanation, however. Firstly, previous work by Röseler (1985) has shown that an increase in growth of the corpora allata (the site of JH production in insects) in *P. dominulus* foundresses is triggered by a rise, rather than a fall, in temperature. If temperature has a similar effect on JH at the pupal stage, we might expect to see more broken patterns at higher temperatures, rather than at lower temperatures. Secondly, and more importantly, if brokenness reflects JH titres, and JH is an important determinant of RHP, then why should brokenness signal RHP in some populations but not in others (see Chapter 3)? Considering again the role of melanin patterns in thermoregulation, it is conceivable that individuals with more black on the clypeus (and elsewhere on the cuticle) might have greater RHP in cooler habitats, where they may be able to sustain energetic behaviours such as fighting at lower temperatures compared with less-melanised individuals. In warmer habitats, conversely, heat absorption is less difficult to achieve, meaning that heavily-melanised individuals no longer enjoy an energetic advantage. Such an effect would constitute a special case of G×E driving variation in the information content of clypeal patterns across different

climatic conditions, with patterns signalling RHP in cold habitats but not in hot habitats, and would also be expected to drive divergence in receiver responses between populations. However, as noted above, there is currently no evidence that individuals with clypeal patterns are more heavily melanised in general, or that differences in melanisation contribute to differences in thermoregulation in this species. Moreover, this hypothesis also fails to explain why RHP is signalled by the amount of disruption in the pattern, rather than simply the amount of black on the clypeus (or on other parts of the body).

5.4.3 Outstanding difficulties

The above discussion of the causes and consequences of temperature-induced variation in clypeal patterning is rendered necessarily speculative by the absence of detailed information on how and why these patterns are expressed. At present, little is known about the factors that influence pattern development in wild populations. Heritability studies indicate that there is additive genetic variation in the amount of black laid down on the clypeus (Tibbetts 2010), but the results of this study indicate that environmental factors can also affect this trait. A major impediment to understanding the development and information content of the clypeus pattern is the ambiguity inherent in the definition and measurement of the active signalling component, brokenness (see Chapter 8). In the context of this study, it is not obvious how the development of clypeal patterning and brokenness in particular should be viewed. Throughout, I have treated the amount of black on the clypeus (i.e. the presence of clypeal patterns) and the brokenness of those patterns as distinct phenotypes, following e.g. Tibbetts 2010. However, it is not clear whether there are two developmental processes governing clypeal pattern development, one determining the presence or absence of melanin and the other determining the specific arrangement of pigment, or whether ‘brokenness’ is determined along a continuous gradient, ranging from no black spots to several spots of varying sizes and shapes. This is important to establish, as it has profound implications for how selection can act on elements of the pattern (see also Chapter 8). For example, does clypeal patterning represent a discrete polyphenism, with the potential for selection to act on a single developmental switch (patterns or no patterns) (Nijhout 1999) or can aspects of

the pattern (size, brokenness) be modelled as a reaction norm, varying continuously along different environmental gradients (Stearns 1989)?

We also know little about the proximate factors driving pattern development, including the period during development in which they exert their influence. Although melanin is laid down only a few days before eclosion (J. P. Green, pers. obs.), sensitivity to factors regulating melanin deposition may occur at an earlier stage of development (Nijhout 1999). In this experiment, only two levels of temperature were used. Although both were within the natural range experienced by developing brood, it is nonetheless likely that variation in temperature during development in the wild will be smaller than the 10°C difference used in this experiment. Small fluctuations in temperature may potentially be important in maintaining variation in clypeal patterning among individuals in the same population (e.g. Tsuruta *et al*. 1989). However, any effect of temperature is unlikely to affect clypeal patterning in isolation; rather, climate may frequently interact with other environmental and genetic factors, resulting in complex patterns of variation within and between populations and locations, which may ultimately be responsible for driving variation in the use of status signals in this species.

5.5 Summary

Research into the cause of within-species variation in sexual signalling has shown that variation in environmental factors can explain variation in male ornaments and variation in female preferences for those ornaments. In this study, I demonstrated that the clypeal patterning in *P. dominulus* is sensitive to temperature during development, This finding provides evidence that signals used in competition can also vary with environmental conditions. Moreover, the results indicate that this variation need not be the result of environmental effects on individual condition, but could instead be the result of plastic responses to local environmental conditions acting to constrain signal expression. Indeed, as appears the case in *P. dominulus*, entire signalling systems may be present or absent from populations depending on variation in environmental conditions.

In Chapters 3 - 5, I explored aspects of intraspecific status signalling in a European population of *P. dominulus*, namely, the signal value of clypeal patterns (Chapter 3); the relationship between clypeal patterning and quality (Chapter 4), and effects of the environment on clypeal pattern development (this chapter). In the following chapter (Chapter 6), I expand the scope of this research to consider signalling and information gathering during interspecific contests. By staging encounters between *P. dominulus* and its social parasite *P. semenowi*, I explore the potential for signalling and rival assessment between species.

Chapter 6: Information gathering in usurpation contests between *Polistes dominulus* and the social parasite *Polistes semenowi*

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6.1 Introduction

6.1.1 Assessment strategies in animal contests

Contests over resources are widespread in nature, both between and within species. The outcomes of contests can have important fitness consequences, not only in terms of the resources gained or lost, but also in terms of the energetic cost of fighting and the risk of injury (e.g. Clutton-Brock *et al*. 1979; Mann *et al*. 2001; Briffa & Sneddon 2007). In order to minimise these costs, individuals may make strategic decisions based on their own resource holding potential (RHP) and that of their rival (Arnott & Elwood 2009). Information acquired during contests about an individual's own performance, as well as its opponent's performance, is valuable as it allows rivals to assess the costs and benefits of continued fighting versus withdrawing from the contest (Arnott & Elwood 2009). Alternatively, or additionally, animals may assess rival ability prior to contests by attending to cues or signals of RHP (Maynard Smith & Harper 2003).

The type of information gathered during contests, as well as the conditions under which information gathering occurs, have been the subject of much theoretical and empirical work (reviewed in Arnott & Elwood 2009). For example, by examining the relationship between winner and loser RHP and contest dynamics, it has been possible to deduce the likely assessment strategies used during contests (Taylor & Elwood 2003; Arnott & Elwood 2009). Information gathering may be limited to an assessment of an individual's own performance during a fight, with individuals withdrawing when an upper threshold is reached (self-assessment). Alternatively, individuals may use information about their own and their rival's RHP (mutual assessment). Furthermore,

information gathered about other aspects of the contest, in particular the value of the contested resource, has been shown to have a critical role in shaping fight dynamics (Arnott & Elwood 2008). Resource value can affect an individual's fighting strategy via its effect on motivation: in cases where resource assessment is possible, individuals that place a greater value on the resource are expected to fight for longer and/or at greater intensities to secure the resource than individuals whose motivation is lower (Arnott & Elwood 2008). Indeed, in situations where the perceived value of the resource is so great that the benefits of winning outweigh the cost of escalated fighting, individuals are expected to persevere, irrespective of asymmetries in RHP (Enquist & Leimar 1990). While there is evidence that high resource value favours escalated fighting over self and rival assessment in certain situations (e.g. Moore *et al*. 2008), in general some form of assessment based on signals/cues or performance during contests is expected to reduce the costs of fighting, and has been demonstrated in a wide range of taxa (e.g. Stuart-Fox 2006; Prenter *et al*. 2008; Tibbetts *et al*. 2010).

6.1.2 Assessment in contests between heterospecifics

To date, studies of animal contests have focused largely on fights between conspecifics for resources. Members of the same species tend to occupy very similar ecological niches and therefore experience intense competition with one another over the limited resources available. Analysis of conspecific contests is aided by the fact that resources valuable to both contestants are readily identified, and factors determining RHP will be common to both contestants. Perhaps for these reasons, contests over resources between members of different species have been largely neglected (but see Tanner & Adler 2009; Cini *et al*. 2011; Pearce *et al*. 2011). However, such contests play an important part in determining an individual's access to resources, with competition occurring between species, for example, for food and breeding sites (e.g. Brown *et al*. 2005; Becerril-Morales & Macías-Ordóñez 2009). Thus, it is important to know which factors influence the outcome of contests between heterospecifics, and to what extent individuals are able to gauge the competitive abilities of heterospecific rivals.

Social parasites of primitively eusocial and eusocial Hymenoptera offer a good opportunity to study assessment strategies used in contests between heterospecifics.

Following invasion of the host nest, social parasites take up residence with the hosts, on whom they depend to rear their offspring. Social parasites deploy a variety of deceptive strategies in order to subvert the host recognition system and so gain entry to the host nest, including chemical mimicry and dilution of cuticular compounds (Lorenzi 2006). However, a number of social parasites in the Vespinae and Polistinae adopt an aggressive usurpation strategy, engaging in prolonged fights with hosts (Reed & Akre 1983; Cervo 2006). While basic elements of these interactions are well described for a number of species (e.g. Reed & Akre 1983; Zacchi *et al*. 1996), there has been little research into the factors determining fight outcome and dynamics, as well as the possible mechanisms of rival assessment used by hosts and parasites during usurpation.

Here, I focus on the aggressive interactions that characterise host usurpation attempts by *Polistes semenowi*, a specialist parasite of the paper wasp *P. dominulus* (Cervo 2006). *P. semenowi* targets host nests in the late pre-emergence phase of the colony cycle, when colony foundresses are the only adults present. The parasite invades aggressively, fighting with host foundresses until they submit (Zacchi *et al.* 1996). The original dominant foundress often remains in the colony, though her role as the principal egg-layer is now assumed by the parasite.

In common with other *Polistes* social parasites, *P. semenowi* presents several morphological specialisations, including enlarged femora and thickened mandibles, which are thought to enhance fighting ability (Cervo 2006). In addition to fighting adaptations, *P. semenowi* also has conspicuous black patterning on the clypeus. In the host species, *P. dominulus*, clypeal patterns play an important role in rival assessment in some populations (Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). In North America, *P. dominulus* patterns function as status signals (Tibbetts & Dale 2004), and there is evidence that they are useful in settling contests between unfamiliar rivals by making asymmetries in RHP apparent when rivals meet (Tibbetts & Lindsay 2008; but see Green & Field 2011b; Chapter 3). The clypeal pattern of the parasite is somewhat larger and less variable than in the host but may similarly function as a signal of status. In the host, the active signal component of the pattern is its disruption, or brokenness (Tibbetts 2010). In the parasite, however, melanin deposition is uniform across the clypeus and extends down to the mandibles (Figure 6.1). Noting this pattern, Ortolani *et al*. (2010)

have alternatively proposed that the parasite clypeal patterns act as ‘amplifiers’ (Taylor *et al*. 2000), highlighting a signal of RHP based on mandibular width. Two recent experiments exploring the signal function of similar patterns in a second *Polistes* parasite, *P. sulcifer*, have yielded contradictory results. Ortolani *et al*. (2010) presented *P. sulcifer* heads to *P. dominulus* hosts, and found that more aggression was directed towards heads painted to conceal the clypeal pattern. Cini *et al*. (2011), however, found that hosts did not respond to similar manipulations during staged contests in the lab. Nevertheless, the finding of Ortolani *et al*. (2010) raises the possibility that the clypeal patterns of *P. semenowi* function in parasite assessment by hosts during usurpation contests. If parasite patterns function as signals of RHP, either as status signals or amplifiers, then they may assist usurpation by reducing host aggressive responses.

Figure 6.1 Portraits of three *P. semenowi* females (top row) with clypeal patterning typical of the species. A single black spot covers the majority of the clypeus and extends down to the jaws. For comparison, portraits of three *P. dominulus* females are presented below (bottom row). As above, each female has a single black clypeal spot, but here there is greater variation in the size and shape of the spot between wasps.

In this study, I explore the potential for rival assessment between *P. semenowi* and *P. dominulus* by experimentally staging host – parasite usurpation contests. I began by testing the hypothesis that parasite clypeal patterns signal RHP during host usurpation by manipulating parasite patterns and observing the effect on the duration and intensity of fights with hosts. Signals of RHP based on clypeal patterns provide a potential mechanism for rival assessment prior to fighting; alternatively (or additionally), individuals may use information about rival RHP acquired during contests when deciding whether to persevere or retreat. Body size in *Polistes* wasps is sometimes associated with social rank (Turillazzi & Pardi 1977; Cervo *et al*. 2008) and in *P. dominulus* body size affects the outcome of both intraspecific (Tibbetts & Shorter 2009) and interspecific (Ortolani & Cervo 2010; Cini *et al*. 2011) usurpation attempts. I therefore also analysed contest dynamics and outcome with respect to rival size in order to establish firstly whether size is a determinant of RHP in *P. dominulus – P. semenowi* contests, and secondly whether size information is used in strategic assessment during fights, as has been reported in other taxa (e.g. Morrell *et al*. 2005; Prenter *et al*. 2008).

6.2 Materials and Methods

6.2.1 Field methods

In order to investigate the dynamics of aggressive interactions between *P. semenowi* and *P. dominulus*, I staged usurpation trials in which a parasite was given the opportunity to usurp a target host nest. To find parasites, I searched for *P. dominulus* colonies parasitised by *P. semenowi* at sites around Conil de la Frontera and Zahara de los Atunes (Cádiz Province, Spain) in early May 2010. Because of the relative rarity of parasites in the study population several hundred colonies had to be inspected in order to obtain the sample of 31 parasites used in the experiment. I removed parasites from their colonies and placed them in individual containers for 24 hours prior to usurpation trials. In order to find suitable target nests, I used intensive field surveys to identify host colonies with no prior history of parasitism. However, it is possible that colonies experienced brief usurpation attempts that were missed by the surveys. Therefore, to ensure that hosts and parasites had not previously interacted, I presented parasites with a

host colony collected at least 3km from where the parasite was found. In order to control for effects of resource value on the motivation of parasites to attack or hosts to defend, I presented parasites with similarly sized target nests, in terms of nest size (mean ± s.e. = 73.16 ± 3.33 cells), number of adult hosts (2.87 ± 0.10), and number of pupae (12.65 ± 1.17).

6.2.2 Parasite manipulation

To test the importance of the parasite clypeal pattern for agonistic interactions with hosts, I randomly assigned parasites to one of two treatment groups (Figure 6.2). In Treatment 1 (n = 14), I added yellow paint to completely cover the black clypeal pattern. In Treatment 2 (n = 17), I added yellow paint to the yellow genae to control for the presence of paint, the odour and spectral reflectance of which is expected to differ from the cuticle. While it is possible that the presence of paint in general alters the behavioural response of the receiver, my experimental design allowed me to test for a specific effect of the parasite clypeal pattern on host behaviour over and above any general response to the presence of paint. Results of a pilot study undertaken in May 2009, in which parasites were painted on the clypeus and genae before being returned to their nests, showed that these manipulations affected neither the parasite's ability to relocate its nest, nor its subsequent behaviour towards hosts (J.P.Green, unpubl. data).

6.2.3 Usurpation Trials

I carried out 31 usurpation trials outdoors on warm, bright days between 1300 and 1600 (identified by Ortolani & Cervo (2009) as the time of peak activity in *P. sulcifer*). In each trial, I placed a single parasite in a plastic cage (34 x 18 x 27cm) containing a target nest and allowed the parasites to approach the nest and interact with hosts (Figure 6.3). In those trials where the parasite approached the nest within two hours, I filmed all fights between parasites and hosts for one hour following the initial approach (henceforth, 'observation period') using a digital camcorder. Each parasite and host colony was used only once in the trials.

6.2.4 Morphological measurements

Upon completion of usurpation trials all individuals were killed by freezing. Following previous studies (e.g. Tibbetts & Dale 2004), I used head width as a measure of body size. For each wasp, I removed the head and placed it on a microscope slide. I measured head width as the width at the widest point using a 16× binocular microscope and graticule. I also recorded the presence or absence of black clypeal spots on the hosts, which is argued to be a good proxy for pattern brokenness (Tibbetts & Lindsay 2008).

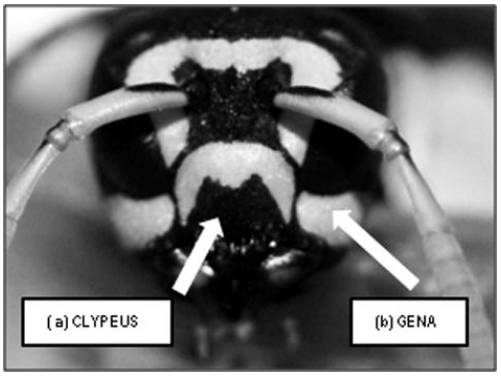

Figure 6.2 Head of *P. semenowi* (♀), with arrows indicating the (a) clypeus and (b) genae painted yellow in Treatment 1 (experimental) and Treatment 2 (control), respectively.

a **b**

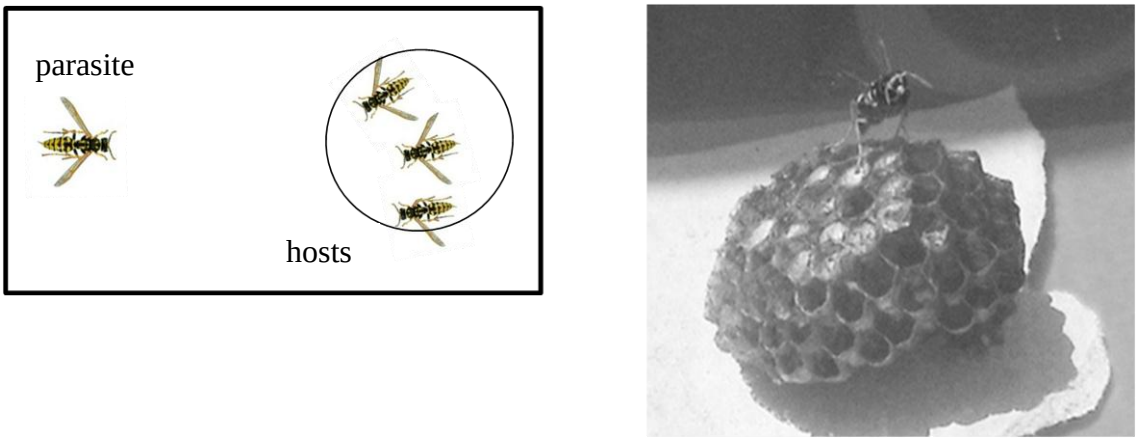

Figure 6.3 Usurpation trial set up. (a) The parasite (left) was released into a transparent plastic box housing a target colony (right). (b) A parasite alone on the nest following a successful usurpation attempt.

6.2.5 Behavioural analyses

For each trial, I recorded the total number of fights within the observation period. I also recorded the duration of each fight, with a pause of more than 30s signifying the end of a fight. To investigate the effect of parasite clypeal patterns on aggressive interactions, I used data only from the initial encounter between parasite and hosts (i.e. the first fight, following the parasite's initial approach to the nest), as it has been suggested that familiarity between signaller and receiver can confound manipulative tests of signal function (Senar 1999; Chapter 3). I obtained three measures from each initial fight: its duration, and two measures of its intensity. To assess fight intensity, I distinguished between two classes of agonistic interactions observed during fights, according to likely energetic costs and risk of injury. 'Low-intensity' interactions were darting, antennating, chasing and lunging (here defined as a rapid movement towards an individual resulting in physical contact). 'High–intensity' interactions were biting, grappling, 'dive-bombing' (a behaviour shown by hosts during usurpation attempts in the wild where hosts fly into the parasite, pushing or biting on contact) and wrestling (wasps clasp legs tightly around one another attempting to sting and/or bite opponent). Fight intensity was then estimated in two ways: the number of high-intensity interactions occurring during the fight, and the total duration of these high-intensity interactions (as a proportion of total fight duration).

6.2.6 Statistical analyses

To test the signal function of the parasite clypeal patterns, I compared the duration and intensity of the first fight between the two treatments. To determine the effect of the paint treatment on fight duration, I used a LM with normal errors. Fight duration (log-transformed) was the response variable and paint treatment was the explanatory variable. Recent work has suggested that, in *P. dominulus*, receiver responses to clypeal patterns depend on the RHP of receivers (Tibbetts *et al*. 2010). Although, in Spain, *P. dominulus* clypeal patterns do not signal status (Green & Field 2011b; Chapter 3), the patterns may still reflect individual RHP, as in other populations. Therefore, in order to test whether host responses to the parasite manipulations were dependent upon host RHP I included the interaction between paint treatment and proportion of hosts with clypeal patterns (hereafter '% hosts with patterns') as an additional explanatory variable

in the analysis. To determine the effect of paint treatment on fight intensity, I compared both the number and duration of high-intensity interactions between treatments. I used a GLM with quasi-Poisson errors to analyse the number of high-intensity interactions, as these data were in the form of overdispersed counts. The duration of high-intensity interactions was analysed as a proportion of the total fight duration. As these data also exhibited overdispersion, they were analysed using a GLM with quasi-binomial errors. Paint treatment was an explanatory variable in both models and, as above, I also included the % hosts with patterns, as well as the interaction between paint treatment and the % hosts with patterns as additional explanatory variables.

To investigate the influence of body size on RHP, I compared the sizes of winners and losers of usurpation contests. Parasites that succeeded in usurping nests were considered winners, and their defeated hosts losers; likewise, parasites that failed to usurp were losers, and their successful hosts winners. Measurements taken from winners and losers cannot be considered as independent because the outcome of a contest (i.e. who wins) is determined by the interaction between the two contestants (Briffa & Elwood 2010). Therefore, to explore the effect of size on contest outcome, I used a LMM with normal errors in which 'trial number' was fitted as a random factor. Following Briffa & Elwood (2010), I fitted size as the response variable and outcome ('winner' or 'loser') as an explanatory variable. In the special case of interspecific contests, it is possible that the extent to which a trait influences contest outcome varies between the two species. To investigate whether the importance of size for contest outcome varies between parasites and hosts, I also fitted the interaction between outcome and contestant species (host or parasite) as an additional explanatory variable.

Finally, I explored the relationship between winner and loser size and contest dynamics to test for possible assessment rules governing usurpation contests. First, I examined the relationship between winner and loser size and the duration and intensity of the first fight. Winners and losers were assigned based on whether the parasite was present on the nest at the end of the fight. To determine the effect of winner/loser size on fight duration, I used a LM with normal errors. Fight duration (log-transformed) was the response variable, and winner and loser size the explanatory variables. As above, fight intensity was analysed as both the number and duration of high-intensity interactions

using GLMs with quasi-Poisson and quasi-binomial errors respectively. Second, I tested for a relationship between winner and loser size and the total number and duration of fights within the 1h observation period. Here, I assigned winners and losers based on whether the parasite was present on the nest at the end of the observation period. The effect of winner-loser size on total duration was analysed using a LM with normal errors. Total duration (log-transformed) was the response variable, and winner and loser size the explanatory variables. Total number of fights did not follow a normal distribution due to many encounters involving only a single fight. I therefore used a GLM with Poisson errors with number of fights as the response variable, and winner and loser size as the explanatory variables.

In all analyses, I calculated host head width as the mean head width of hosts within colonies. Substituting mean host head width for the head width of the largest host did not alter the results. Means are reported ± 1 s.e.

6.3 Results

6.3.1 General features of usurpation fights

Usurpation attempts by the parasite (observed in 24/31 trials) were characterised by one or more approaches towards the host nest, resulting in physical fighting with the hosts. The initial fight continued until the parasite retreated (11/24 trials), or until the hosts withdrew, leaving the parasite alone on the nest (13/24 trials). Parasites that retreated after the initial fight were observed to make further approaches to the nest in 6/11 trials. Where parasites succeeded taking over the nest after the initial fight, hosts returned and engaged the parasite in further fighting in 8/13 trials. At the end of the observation period (1h after the initial approach), 14 parasites were on the nest and 10 parasites were off the nest.

6.3.2 Effects of host and parasite clypeal patterns

For those trials in which parasites approached the nest, I examined the influence of the clypeal pattern manipulation on the dynamics of the ensuing fight. Duration of the initial fight was not influenced by the pattern manipulation (paint treatment: $F_{1,23}$ = 0.35, p = 0.56; treatment x % hosts with patterns: $F_{3,23}$ = 1.63, p = 0.21; Figure 6.4). Independent of the pattern manipulation, fight duration tended to be positively related to the proportion of hosts with clypeal patterns, though this was not statistically significant ($F_{1,23}$ = 3.56, p = 0.07). Manipulation of the clypeal pattern also had no effect on the intensity of the first fight, in terms of either the frequency of high-intensity interactions ($F_{1,23}$ = 0.31, p = 0.58; treatment x % hosts with patterns: $F_{3,23}$ = 1.10, p = 0.37) or the proportion of time spent engaged in high-intensity interactions ($F_{1,23}$ = 0.04, p = 0.85; treatment x % hosts with patterns: $F_{2,23}$ = 0.11, p = 0.90). Again, however, there was an independent effect of host clypeal patterns: relatively more time was spent engaged in high-intensity interactions when a higher proportion of hosts had clypeal patterns ($F_{1,23}$ = 6.55, p = 0.02). The proportion of hosts with clypeal patterns tended to also be positively associated with the frequency of high-intensity interactions, though this was not statistically significant ($F_{1,23}$ = 3.60, p = 0.07).

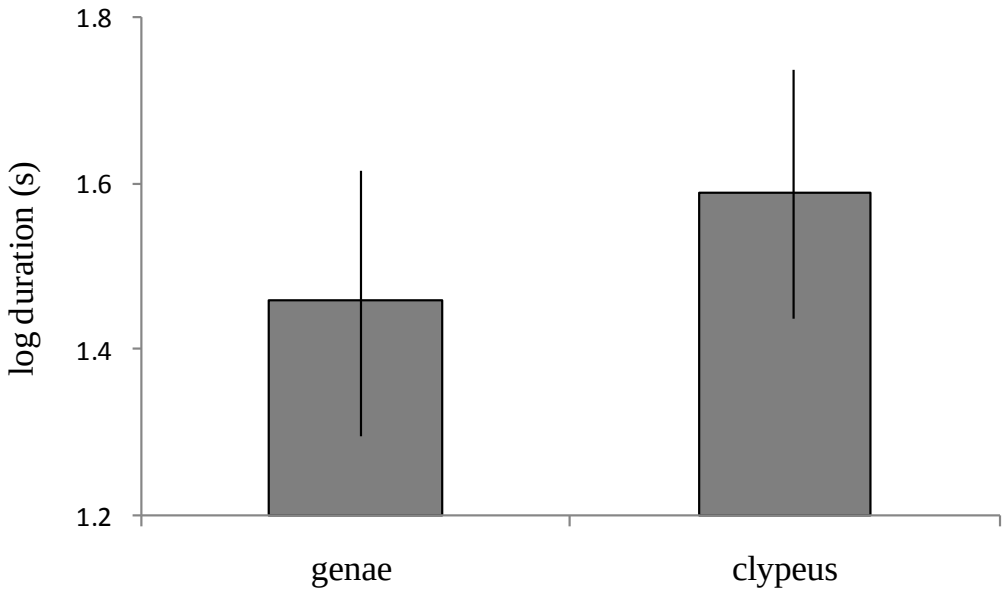

Figure 6.4 Duration of initial fights (n = 24) in relation to parasite manipulation (paint concealing the genae or clypeus). Means are shown ± 1 s.e.

6.3.3 The role of body size in RHP

To explore the role of body size in RHP, I compared the sizes of winners and losers. Winners of the first fight (i.e. the fight following the initial approach by the parasite) were not significantly larger than losers (3.57 ± 0.02 vs. 3.54 ± 0.03 mm; $L_1 = 0.86$, $p = 0.35$). However, in a second comparison of winner and loser size, this time based on whether nests had been usurped by the end of the observation period (i.e. 1h after the first interaction), winners were significantly larger than losers (3.59 ± 0.02 vs. 3.51 ± 0.02 mm; $L_1 = 5.45$, $p = 0.02$; Figure 6.5). There was no significant interaction between outcome and species, indicating that the importance of size in determining fight outcome does not vary between species ($L_1 = 2.10$, $p = 0.35$).

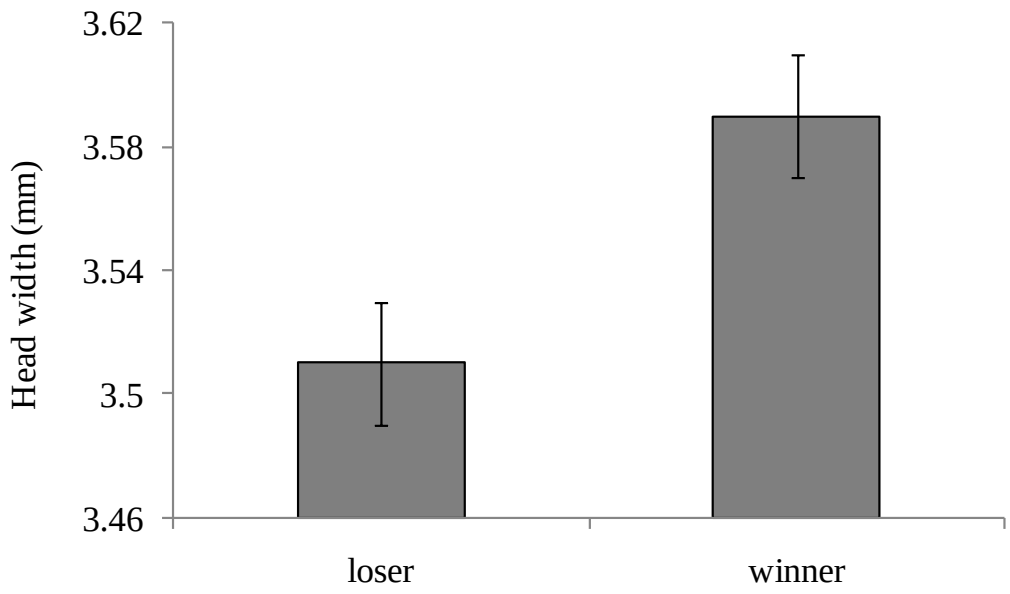

Figure 6.5 Mean head width of winners and losers, based on whether the parasite was on the nest at the end of the observation period ($n = 24$ fights). Means are shown ± 1 s.e.

Two processes could account for the increase in size difference between winners and losers over the observation period. First, larger parasites that initially failed to usurp the nest may win against hosts in further fights. Although small sample sizes preclude statistical analysis, a comparison of mean head widths suggest this might be the case: average head width of parasites that subsequently usurped the nest was greater than that

of parasites that lost the first fight and did not ultimately manage to usurp nests (3.69 ± 0.06 vs. 3.52 ± 0.05 mm, n = 3 and 8 respectively). Additionally, or alternatively, smaller parasites may be more vulnerable to eviction from the nest following initial usurpation. Average head width of parasites that were evicted from the nest did not differ from that of parasites who retained the nest (3.55 ± 0.09 vs. 3.56 ± 0.04 mm, n = 2 and 11, respectively), although here again sample sizes are small.

6.3.4 Body size in rival assessment

The importance of body size as a determinant of contest outcome indicates that information about rival size may be useful to individuals when choosing whether to persevere or retreat. To test for possible assessment rules, I analysed the relationships between contest dynamics and the size of winners and losers. Focusing on the first fight, I found no significant relationship between fight duration and the size of winners ($F_{1,23}$ = 0.00, p = 0.99) or losers ($F_{1,23}$ = 0.00, p = 0.95). I also found no relationship between the intensity of the first fight and the size of winners or losers, whether considering the frequency (winners: $F_{1,23}$ = 0.05, p = 0.82, losers: $F_{1,23}$ = 0.16, p = 0.70) or duration (winners: $F_{1,23}$ = 0.16, p = 0.69; losers: $F_{1,23}$ = 0.01, p = 0.93) of high-intensity interactions. I also looked for evidence of rival assessment across all fights. Total duration of all fights that occurred within the observation period was not significantly predicted by either winner size ($F_{1,23}$ = 0.67, p = 0.42) or loser size ($F_{1,23}$ = 0.20, P = 0.66). Likewise, the total number of fights was not significantly predicted by either size of winners (χ^2_1 = 1.62, p = 0.20) or losers (χ^2_1 = 2.27, p = 0.13).

6.4 Discussion

6.4.1 The role of host and parasite clypeal patterns

I found no evidence that clypeal patterns in the social parasite *P. semenowi* function to reduce host aggression during nest usurpation. Previous research into rival assessment in *P. dominulus* has emphasised the importance of clypeal patterns as signals of RHP in settling contests between unfamiliar individuals (Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). Although there is no evidence for intraspecific status signalling via clypeal

patterns in European populations (Green & Field 2011b; Chapter 3), results from a recent experiment have suggested that such patterns may be important in interspecific contests (Ortolani *et al*. 2010). Presentation experiments using heads of the related parasite *P. sulcifer* demonstrated an increase in host aggression towards parasite heads manipulated to conceal the clypeal pattern (Ortolani *et al*. 2010; but see Cini *et al*. 2011). This finding raises the possibility that parasite clypeal patterns could function as interspecific status signals, exploiting a mechanism of conflict resolution among hosts in order to dissuade hosts from aggressive retaliation during nest usurpation. Alternatively, the parasite's patterns may function as amplifiers, highlighting the width of the mandibles (Ortolani *et al*. 2010). In my experiment, which simulated real usurpation contests between *P. dominulus* hosts and the social parasite *P. semenowi*, concealment of the pattern did not result in greater aggression from hosts, in terms of either fight duration or intensity. In addition, a non-significant interaction between treatment and the proportion of hosts with clypeal marks showed that this response to the paint treatment was not dependent on hosts' clypeal patterns. My results therefore suggest that *P. semenowi* clypeal patterns are not used in rival assessment during nest usurpation, either as status signals or amplifiers.

However, it is important to emphasise the relatively small sample sizes in this study, necessitated by logistical considerations (i.e. the relative rarity of parasites in the population). The size of the effect of paint treatment on fight duration, calculated as Cohen's *d*, was 0.24 with 95% confidence interval (CI) of -0.56 – 1.05 (note that this is on a log scale). Thus, for the analysis to detect a significant effect of the manipulation on fight duration, the effect size would need to be large (i.e. > 1.05 standard deviations). Similar effect sizes would also be necessary to detect a significant effect of the manipulation on fight intensity. (Effect sizes were calculated using the online calculator at http://gunston.gmu.edu/cebcp/EffectSizeCalculator/index.html, as recommended by Nakagawa & Cuthill [2007]). However, it is worth noting that another study using the Spanish population and employing a larger sample size likewise found no evidence that hosts react to rival clypeal patterns, this time during interactions with conspecifics (Green & Field 2011b; Chapter 3). Below, I discuss the difference in results between this study and that of Ortolani *et al*. (2010) in terms of the context-dependence of

receiver behaviour and the costs and benefits of rival assessment during usurpation contests (see 6.4.3 'Limitations on rival assessment').

Although parasite clypeal patterns play no role in usurpation contests, host clypeal patterns do influence fight dynamics: initial fights were significantly more intense and also tended to be longer in trials where a greater proportion of defending hosts had clypeal patterns. One explanation for this is that hosts with clypeal patterns have higher RHP and are better able to resist the invading parasite, investing energy in prolonged and high-intensity defence behaviours (Tibbetts & Shorter 2009), though again it has been shown in other work that clypeal patterns do not communicate this information to conspecifics (Green & Field 2011b; Chapter 3). A second explanation is that parasites are more motivated to attack colonies of patterned hosts because such colonies are more attractive to the parasite, leading to longer and more intense fights. Previous work has shown that *Polistes* social parasites preferentially target larger nests with mature brood (Cervo & Turillazzi 1996; Shreeves *et al*. 2003). If host clypeal patterns reflect aspects of quality associated with greater colony size and productivity (e.g. survival, fecundity), then parasites able to assess host clypeal patterns, or traits correlated with patterning, might be expected to target high-quality hosts preferentially (but see Chapter 4). Further research into the characteristics of host colonies targeted by the parasite is needed to determine which host traits are favoured by *P. semenowi*, as well as how these traits are assessed by the parasite during host selection.

6.4.2 The importance of body size

The analysis revealed the importance of opponent size on outcome of parasite usurpation attempts. Larger parasites were more likely to successfully usurp nests, while larger hosts were more successful in defending nests. Body size has often been suggested as a potential determinant of RHP in *Polistes* wasps (e.g. Turillazzi & Pardi 1977; Cervo *et al*. 2008). In particular, previous studies have indicated that body size influences both intraspecific (Tibbetts & Shorter 2009) and interspecific (Ortolani & Cervo 2010; Cini *et al*. 2011) usurpation contests. Ortolani & Cervo (2010) examined geographic variation in *P. dominulus* body size in relation to parasitism by *P. sulcifer*. The authors found that larger hosts were more active in fighting a usurping parasite, and

were consequently less likely to be parasitised (Ortolani & Cervo 2010). Furthermore, the occurrence of larger hosts in populations experiencing high rates of parasitism led the authors to suggest that larger host body size may be the result of a parasite-host arms race, in which large body size is selected as a defence against parasite invasion (Ortolani & Cervo 2010). My result suggests that a similar relationship may be found between *P. dominulus* size and *P. semenowi* density; however, more data on host size and parasitism rates from different areas are needed to test this hypothesis.

Importantly, the finding that size influences the outcome of usurpation contests raises the possibility that information about opponent size is used in decision-making during competitive interactions. To investigate whether size-based assessment occurs during usurpation contests, I analysed the relationship between duration and intensity of the first fight, as well as the total number and duration of fights within the observation period, and the size of winners and losers (as judged by usurpation success/failure). Looking within the first fight, I found that neither duration nor intensity was significantly predicted by the size of winners or losers. Furthermore, within the whole observation period, neither total fight duration nor the total number of fights were significantly predicted by either loser or winner size. Again, however, I draw attention to the relatively small sample sizes available in this study. Calculation of effect sizes indicates that moderate to large effects of size on fight measures could be detected with my sample size. For example, the size of the effect of loser size on contest duration (partial correlation coefficient, r) is -0.1 with 95% CI -0.48 – 0.32 (on a log scale), and the other effects were of similar sizes.

The absence of a positive correlation between loser size and measures of fight duration and/or intensity in particular argues against assessment by rivals during usurpation. A common prediction of all models of assessment is that contest duration and/or intensity should increase with increasing loser RHP (Arnott & Elwood 2009). This is true for strategies of self assessment, where the decision to withdraw is taken when some cost threshold, determined by individual RHP, is exceeded (Taylor & Elwood 2003). In the case of mutual assessment, a positive relationship between loser RHP and duration and/or intensity is also expected, given that the decision to withdraw versus escalate is based on RHP asymmetry between contestants, which is most easily perceived when

differences in RHP are large (Taylor & Elwood 2003). The absence of a positive relationship between loser size and duration or intensity in this study would therefore appear to rule out assessment during usurpation contests. Thus, while larger body size confers greater RHP, there is no evidence that size assessment is used to minimise fight costs during usurpation attempts.

6.4.3 Limitations on rival assessment

The ability to use information about opponent RHP to make tactical decisions during fights is often considered advantageous as it allows individuals to avoid the costs (e.g. injury, depletion of energy reserves) of escalated conflict. Given these benefits, our finding that wasps do not use information about rival RHP is somewhat surprising. A possible explanation for the apparent lack of assessment is that both hosts and parasite are highly motivated to fight on account of the high value of the contested resource. From the hosts' perspective, usurpation of a colony by a social parasite represents a potentially fatal assault on host fitness. Upon usurpation, the parasite destroys younger brood, preserving older brood as a workforce to rear its offspring (Cervo 2006). Combined with high reproductive skew in favour of the parasite (Chapter 7), this often results in a dramatic reduction in host fitness following parasitism (e.g. Lorenzi *et al*. 1992). Although *Polistes* foundresses are known to build new nests following nest predation (Strassmann *et al*. 1988), the extent to which this is a viable option for parasitised hosts is unknown. Usurpation by social parasites occurs at a relatively late stage in the season (i.e. just prior to worker emergence); opportunities for re-nesting may therefore be time-limited. However, the potential for re-nesting is likely to influence perceived nest value during host-parasite contests and therefore merits further study, particularly as there is evidence that hosts do occasionally abandon nests following successful usurpation by parasites (Almond 2007).

From the perspective of the parasite, which is dependent on a host workforce for offspring production, successful usurpation is critical. Furthermore, parasites enjoy only a brief window in which to attack, in the late pre-emergence period of the host nest cycle (Cervo 2006), which would be expected to limit the number of usurpation attempts a parasite can make, whether on the same nest or on different nests. No direct

evidence about multiple usurpation attempts by *P. semenowi* in the wild is available at present, and would require both tracking of individual parasites and detection of all usurpation attempts, which are sometimes very brief (J.P. Green, pers. obs.).

Thus, in the case of usurpation contests, the fitness payoff associated with successfully usurping or successfully defending the nest may in fact exceed any costs associated with escalated fighting (Enquist & Leimar 1990), thereby negating any benefit of rival assessment. Furthermore, given the enhanced weaponry of *Polistes* social parasites (Cervo 2006), any respect shown for RHP asymmetries during usurpation fights would very often, if not always, result in acceptance of the parasite by the host colony, and the fitness costs associated with parasitism. In the face of these 'divisive' asymmetries (Grafen 1987), the optimal strategy may be to persevere irrespective of asymmetries in RHP until the costs of injuries and depletion of energy reserves force retreat (Grafen 1987). Support for this scenario comes from observations (this study) of serious injuries sustained to both hosts and parasites during trials, including the loss of legs and damage to wings. Although such injuries impose significant costs, escalated fighting of the kind I observed may still be favoured if such costs are outweighed by the cost of losing the nest (Enquist & Leimar 1990; Elias *et al*. 2010).

High resource value may also explain why I found no evidence of rival assessment based on clypeal patterns. The use of signals or cues of RHP is argued to facilitate assessment prior to fighting, thereby minimising costs of conflict (Maynard Smith & Harper 2003). However, such signals may have limited value in contexts where resource value is high (Maynard Smith & Harper 1988; Tibbetts 2008; but see Számadó 2011). As discussed, nest usurpation may be one context in which the value of the resource actually exceeds the cost of fighting; RHP signals may therefore be of limited importance in these contests. In the experiments by Ortolani *et al*. (2010), which demonstrated an effect of parasite clypeal patterns on host aggression, receiver responses were not tested in the context of nest usurpation. In both cases where the signal value of clypeal patterns was examined during usurpation (Cini *et al*. 2011; this study) patterns did not affect host aggressive behaviour. In a separate observational study of intraspecific nest usurpation in *P. dominulus*, usurpation success was predicted by the relative clypeal patterning of the intruder and resident (Tibbetts & Shorter 2009).

However, signal manipulation experiments testing whether patterns alone determined contest outcome were not performed. If, in real usurpation attempts, the motivation for intruders (conspecifics or social parasites) to attack and for hosts to defend is high, then selection may favour escalation over information gathering that allows tactical retreat based on opponent assessment (Enquist & Leimar 1990; Moore *et al*. 2008).

6.5 Summary

This study aimed to investigate potential assessment rules guiding interspecific contests between a usurping social parasite and its hosts. I found no evidence that parasite clypeal patterns function to reduce host aggression during contests. Larger parasites were more successful in usurping nests, and larger hosts more successful in defence, but assessment based on size does not appear to occur during contests. One explanation for this result is that the value of the resource to both contestants outweighs any risks associated with escalated fighting. Together with the prospect for divisive asymmetries during contests, selection may therefore favour all-out fighting over rival assessment in these contests.

In this chapter I focused on the scope for information gathering during initial host – parasite encounters. In the following chapter (Chapter 7), I revisit the social parasite *P. semenowi* after it has established itself among its hosts as the new reproductive dominant. I explore the factors affecting competition over reproduction between hosts and parasites in order to gain an insight into the general mechanisms of reproductive partitioning in animal societies.

Chapter 7: Social parasitism and reproductive skew in the primitively-eusocial wasp *Polistes dominulus*

7.1 Introduction

7.1.1. An introduction to reproductive skew theory

The partitioning of reproduction between individuals in animal societies has long been a topic of intense interest and debate (Keller & Reeve 1994; Clutton-Brock 1998; Nonacs 2006; Field & Cant 2009). Reproductive partitioning (or reproductive 'skew') in cooperatively-breeding groups can vary dramatically both within and between species, ranging from an equal distribution of reproduction among all group members (low skew) through increasingly unequal reproduction to a complete monopoly by a single individual (high skew). In recent decades, efforts to understand this variation have centred around tests of competing 'reproductive skew' models (e.g. Field *et al*. 1998; Reeve *et al*. 2000; Seppä *et al*. 2002; Langer *et al*. 2004; Liebert & Starks 2006). These models aim to explain skew in reproduction in terms of negotiations and/or competition over reproductive benefits, the nature and outcome of which are shaped by various social and ecological factors, including kinship, resource-holding potential (RHP), group productivity and constraints on independent breeding (for reviews see Johnstone 2000; Reeve & Keller 2001; Cant & Field 2009). While all models share a basic stability condition under which group formation is favoured over solitary breeding, they differ in their assumptions about how the allocation of reproduction among group members is controlled and, in doing so, generate different predictions about the relationship between reproductive skew and social and ecological factors that can be tested against empirical data (Reeve & Keller 2001).

A fundamental question when studying reproductive skew is how allocation of reproduction is controlled within a group. Skew models differ in their assumptions about which individual(s) control(s) reproduction: transactional models postulate that one individual has complete control of reproduction, while compromise models assume no one individual has complete control over reproduction (Johnstone 2000).

Transactional models can be further divided into two types: concessions models and restraint models, both of which assume that skew is the outcome of a negotiation between individuals but which differ in their assumption about who controls reproduction. Concessions models assume that the dominant (defined as the individual that controls group membership) controls reproduction, and that skew is determined by the amount of reproduction that the dominant allocates to the subordinate in order to retain it peacefully in the group. Thus, the 'concession' (Clutton-Brock 1998) offered by the dominant to the subordinate can be seen as consisting of two parts: a 'staying incentive' sufficient to prevent the subordinate from leaving the group to pursue independent reproduction (Vehrencamp 1983), and a 'peace incentive' sufficient to prevent the subordinate mounting a challenge for the dominant position and the associated reproductive benefits (Reeve & Ratnieks 1993). Basic formulations of the concessions model (e.g. Johnstone 2000) predict that the magnitude of the concession will vary with dominant-subordinate relatedness, subordinate RHP relative to the dominant, group productivity and the constraints on independent breeding by the subordinate. Specifically, concessions models predict that the subordinate's incentive will be smaller (i.e. skew will be higher) when relatedness is high, because the subordinate receives compensation in the form of greater indirect benefits from helping to rear the dominant's relatives. Concessions are also predicted to be small when productivity and constraints on independent breeding are high, as in both cases subordinates obtain greater benefits by remaining in the group rather than departing. Finally, concessions will be smaller when subordinate RHP is low, as the threat of a successful challenge to the dominant is reduced (Reeve & Ratnieks 1993).

In contrast to concessions models, restraint models assume that, while the dominant retains control of group membership, a subordinate is free to determine its own share of reproduction provided that it does not exceed a threshold beyond which it becomes unprofitable to the dominant to retain the subordinate and the group dissolves (Cant 1998). Although both concessions and restraint models seek to find levels of skew that are needed to ensure group stability, their assumptions about who controls reproduction lead to exactly opposite predictions (Johnstone 2000). Restraint models thus predict that a subordinate's share of reproduction increases (i.e. skew decreases) when relatedness is high because the dominant is expected to tolerate more reproduction by a related

subordinate, given the indirect benefits it accrues. Likewise, when productivity and constraints on independent breeding are high, subordinates are expected to obtain more reproduction as, under these conditions, the dominant favours retaining the subordinate over evicting it. Although the original restraint model of Cant (1998) does not consider the effect of subordinate RHP, it might be predicted that skew decreases with increasing subordinate RHP as in the concessions model, because the risk associated with forcibly evicting a subordinate presumably increases with the subordinate's RHP.

Both concessions and restraint models assume complete control of reproduction by one individual; in compromise (or 'tug-of-war') models, however, it is assumed that neither individual has complete control over reproduction. Rather, skew is determined through competition between individuals, with RHP asymmetries determining the share of reproduction each individual receives (Reeve *et al*. 1998). Unlike transactional models, in which some reproduction is offered as a concession to ensure group stability, tug-of-war models assume that all reproduction may be contested, and thus do not consider the possibility of group dissolution (leaving or eviction) in response to the partitioning of reproduction following competition (Reeve *et al*. 1998). Consequently, factors such as productivity and constraints on independent breeding that affect an individual's decision to join a group are predicted to have no effect on skew in a tug-of-war over reproduction (Johnstone 2000). Rather, given the role of competition in determining reproduction, asymmetries in RHP are important in determining skew, with high skew predicted when subordinate RHP is low. Unlike transactional models, in which relatedness has a pronounced effect on skew, the original tug-of-war model of Reeve *et al*. (1998) predicts either no relationship between relatedness and skew, or else a weakly negative relationship when subordinate RHP is very low (however, other compromise models make different predictions; see Johnstone 2000).

As can be seen in the brief summary above, transactional and tug-of-war models make different predictions about the relationship between skew and various social and ecological factors, reflecting their assumptions about how reproduction within groups is allocated. For instance, while tug-of-war models predict no relationship between skew and relatedness, concessions models predict that high skew should be found in groups of close relatives. One approach to distinguishing between these models has been to

compare the predictions of different models against observed patterns of skew (e.g. Field *et al*. 1998; Seppä et al. 2002; Koenig *et al*. 2009). However, this approach has been complicated in recent years by the proliferation of new models which seek variously to incorporate new parameters relevant to social living and synthesise elements of traditional models in an effort to provide a more realistic description of the processes underpinning reproductive partitioning (e.g. Kokko & Johnstone 1999; Johnstone 2000; Reeve & Emlen 2000). For example, extending the basic concessions model, Kokko & Johnstone (1999) showed that the opportunity for future reproduction via inheritance of the dominant position reduces the concession required by a subordinate to remain in the group, such that high skew is possible even within groups of unrelated individuals (Kokko & Johnstone 1999; see also Leadbeater *et al*. 2011). In the N-person model, Reeve & Emlen (2000) explore factors affecting the size of staying incentives in groups of varying size, and distinguish between groups that are unsaturated and saturated with subordinates. In unsaturated groups, skew is determined by relatedness, productivity and breeding constraints as in the traditional concessions model. In saturated groups, however, the magnitude of subordinate incentives can vary independently of relatedness, such that relatedness is not always a reliable indicator of skew in saturated groups (Reeve & Emlen 2000).

7.1.2. Tests of reproductive skew models

Genera of primitively eusocial Hymenoptera including *Polistes* have provided an important testing ground for skew models due to the small size of groups, the scope for all individuals to pursue independent reproduction, and the development of molecular techniques permitting the accurate assignment of offspring to individual adults (Field *et al*. 1998; Reeve *et al*. 2000; Paxton *et al*. 2002; Seppä *et al*. 2002; Sumner *et al*. 2002; Langer *et al*. 2004; Liebert *et al*. 2005; Liebert & Starks 2006; Fanelli *et al*. 2005; Lucas *et al*. 2011). In primitively eusocial bees and wasps, subordinate control of reproduction is considered unlikely, based on the small group sizes and presence of the dominant at the nest for long periods of time, which would make it difficult for subordinates to reproduce without detection (Field & Cant 2009). Tests of skew models in these taxa have thus tended to focused on concessions and tug-of-war models, where the dominant is assumed to have either complete or partial control of reproduction.

Somewhat surprisingly, given the focus on primitively eusocial taxa in the development of skew theory (e.g. Reeve & Ratnieks 1993), empirical support for either model has been limited. An important difference between concessions and tug-of-war models is the predicted effect of relatedness on skew. Evidence of a positive correlation between relatedness and skew, consistent with reproductive concessions, is limited to three studies (*Lasioglossum malachurum*: Paxton *et al*. 2002; *Microstigmus nigropthalmus*: Lucas *et al*. 2011; *P. fuscatus*: Reeve *et al*. 2000), while evidence of a negative correlation, predicted by some tug-of-war models, is limited to two studies (*Exoneura nigrescens*: Langer *et al*. 2004; *P. bellicosus*: Field *et al*. 1998). Rather, the most common finding is that skew does not vary with relatedness (*Liostenogaster flavolineata*: Sumner *et al*. 2002; *Parischnogaster mellyi*: Fanelli *et al*. 2005; *P. aurifer*: Liebert *et al*. 2005; *P. carolina*: Seppä *et al*. 2002; *P. dominulus*: Queller *et al*. 2000; Liebert & Starks 2006; Leadbeater *et al*. 2011). Although predicted by certain variants of the incomplete control model, the absence of a relationship between skew and relatedness is not by itself convincing evidence for a tug-of-war over reproduction, as it could also occur in situations where skew is determined by a simple convention, such as age or tenure within the group (e.g. Seppä *et al*. 2002). Instead, the critical prediction of tug-of-war models is that skew should decrease with increasing subordinate RHP. To date, however, there is no evidence that skew varies with subordinate RHP, commonly measured as body size (Field *et al*. 1998; Seppä *et al*. 2002; Lucas *et al*. 2011).

The lack of support for the predictions of concessions and tug-of-war models does not necessarily imply, however, that such models are without value (cf. Nonacs 2006). Rather, the failure of previous studies to support or reject predictions of skew models may be due, in part, to limitations of the data sets collected by those studies. A recent review by Field & Cant (2009) argued that, in many cases, the data against which competing skew models have been tested have lacked sufficient power to detect relationships between skew and other variables. The lack of power can be attributed to both the small sample sizes in many studies (<30 groups in studies of skew in wasps; Field & Cant 2009), and the often uniformly high levels of skew across groups. The lack of variation in skew in particular poses problems when testing for associations between skew and social and ecological factors, though the persistence of high skew may suggest that reproductive partitioning is in fact relatively insensitive to these

factors. Further exacerbating the problem of low power in tests of skew models is the low variability in relatedness between groups observed in many species (Field & Cant 2009). The relationship between skew and relatedness is an important distinguishing feature of concessions and tug-of-war models, yet the high percentage of groups containing close relatives reported in some studies (e.g. Field *et al*. 1998; Seppä *et al*. 2002; Sumner *et al*. 2002) may have reduced their power to detect this relationship. A final problem highlighted by Field & Cant (2009) concerns the estimation of subordinate RHP, which is of fundamental importance in determining skew in tug-of-war models, but is also important in determining the size of the peace incentive in concessions models. In particular, it is possible to question the validity of equating RHP with body size, a common practice in studies of skew in *Polistes* and other wasp genera (e.g. Field *et al*. 1998; Seppä *et al*. 2002; Lucas *et al*. 2011). In many cases, there is often no a priori reason for expecting size to confer a competitive advantage; indeed, across primitively eusocial wasps as a whole, body size appears to be at best an unreliable indicator of dominance (Field & Cant 2009; Chapters 4 & 6). Crucially, without precise knowledge of the determinants of RHP in a particular species, it is hard to test how skew varies with competitive ability, with the result that the importance of competition may be underestimated in a number of studies.

7.1.3. Reproductive skew in *Polistes dominulus*

In this study, I aim to explore reproductive skew in co-foundress groups of the paper wasp *P. dominulus*. Previous studies of reproductive partitioning in this species have concluded that reproductive concessions are unlikely to explain the levels of skew in co-foundress groups, based on the observation that skew does not vary with intra-group relatedness (Queller *et al*. 2000; Liebert & Starks 2006; Nonacs *et al*. 2006). The absence of a relationship between skew and relatedness could suggest that skew is instead determined by a tug-of-war over reproduction. However, the central prediction of the tug-of-war model – that skew should decrease with increasing subordinate RHP – has not been tested. Results to date therefore indicate that competition over reproduction could perhaps be important in determining skew in *P. dominulus*, but that concessions models cannot explain patterns of reproductive partitioning (Nonacs *et al*. 2006).

There are at least two reasons, however, for approaching these results with caution. First, of the two studies arguing against reproductive concessions, one (Liebert & Starks 2006) looked at only 10 nests, which therefore limited the power of the study to detect a statistical effect of relatedness on skew. Second, the finding that relatedness and skew are not correlated may not be sufficient to reject concessions models where individuals are unable to respond directly to variation in relatedness (Field & Cant 2009). While *P. dominulus* co-foundress groups contain significant numbers of unrelated individuals (Queller *et al*. 2000; Liebert & Starks 2006; Zanette & Field 2008), it is unclear whether individuals can detect and respond to this variation (Dapporto *et al*. 2004; Gamboa 2004). If relatedness cues are unavailable, estimates of kinship may instead be based on whether individuals shared the same natal nest in the previous year and on the mean relatedness to natal nest-mates (Gamboa 2004). The lack of a relationship between skew and relatedness does not therefore necessarily rule out reproductive transactions if reproduction is allocated between relatives based on general rules of thumb, rather than in direct response to variation in relatedness (Field & Cant 2009). One solution to this problem may be to artificially generate variation in intra-group relatedness that group members are able to detect. For instance, if *P. dominulus* foundresses use former nest-mate cues as a proxy for relatedness, skew models could be tested by comparing skew between artificially-created groups of former nest-mates and former non-nest-mates, following the approach of Langer *et al*. (2004). Unfortunately, however, attempts to manipulate group formation in this way have so far met with little success (E. Leadbeater & J. Field, unpublished data). Ultimately, the failure of previous studies to consider possible difficulties in discriminating relatedness, together with limitations arising from small sample sizes and low power, means that a convincing test of reproductive transactions in *P. dominulus* is still lacking.

7.1.4 Reproductive skew: insights from social parasites

Thus far, I have considered reproductive skew in terms of reproductive partitioning within groups of conspecifics. Cooperative breeding among conspecifics is by far the most common pattern observed in nature; heterospecific breeding associations, in contrast, are relatively unusual and typically associated with exploitation of a host species by a second, parasitic species. In the social Hymenoptera, a number of species

reproduce via a strategy known as social parasitism, whereby a female gains control of reproduction within a host colony and her offspring are then reared by host workers (D'Ettorre & Heinze 2001; Cervo 2006). In the genus *Polistes*, three obligate socially parasitic species are known (Cervo 2006; Chapter 1). Though the tactics used to infiltrate the nest and subdue hosts vary between the species, the end result is the same in all cases: once on the nest, the parasite assumes the position of reproductive dominant and proceeds to lay eggs that are reared by the subordinate hosts (Cervo 2006). Previous authors have studied the partitioning of reproduction among *Polistes* social parasites and their hosts within parasitised colonies in the context of the natural history and evolution of parasitism within the genus (e.g. Lorenzi *et al*. 1992). However, as I will argue below, social parasitism also offers a valuable occasion to address questions relating to the evolution of cooperative breeding, and reproductive skew in particular.

In this study, I focus on the reproductive partitioning between the host *P. dominulus* and the social parasite *P. semenowi* (Figure 7.1). *P. semenowi* targets host colonies in the late pre-emergence phase of the host colony cycle and fights aggressively with hosts for control of the nest (Zacchi *et al*. 1996; Chapters 1 and 6). Upon the replacement of the dominant host by the parasite, there is a significant shift in two parameters that are of central importance in skew theory. The first shift is seen in the relatedness between a subordinate and its dominant. On unparasitised nests, relatedness between a subordinate and a dominant is often (though not always) positive. Following parasitism, however, relatedness between a subordinate and its new dominant is now zero, as dominant and subordinate are different species. The second important shift occurs in the subordinate's RHP relative to the dominant, which is expected to decrease significantly following parasitism. This is due to the presence of various morphological specialisations in the parasite, including larger body size and thickened mandibles, which are thought to have evolved as adaptations to violent usurpation of host colonies , conferring on parasites a greater RHP than is seen in hosts (Cervo 2006).

Parasitised and unparasitised colonies can therefore be viewed as two distinct types of social group, one where dominant-subordinate relatedness is low and subordinate RHP is low, and the other where relatedness is high and subordinate RHP is high. By comparing skew between parasitised and unparasitised colonies, it is therefore possible

to explore the relative importance of negotiations versus competition (i.e. concessions versus tug-of-war) in determining skew in reproduction in *P. dominulus*. If skew is determined via concessions, skew is expected to be lower in parasitised colonies than in unparasitised colonies due to the low relatedness between subordinates and their parasite, which prevents them from obtaining indirect benefits and leads them to demand a greater share of direct reproduction. If, however, skew is determined by a tug-of-war over reproduction, skew should be higher in parasitised colonies due to the greater asymmetry in RHP between subordinates and parasites than between subordinates and dominants in unparasitised colonies.

Figure 7.1 *P. dominulus* co-foundress nest parasitised by *P. semenowi*. Here, the parasite (marked with pink paint) can be observed laying an egg.

Social parasitism can thus be seen to represent a 'natural experiment', in which the predictions of skew models can be tested by comparing skew in parasitised and unparasitised colonies. This approach requires some justification. In particular, is it appropriate to draw conclusions about how reproductive skew is determined within a species using comparisons with heterospecific, parasitic associations? The answer to this depends upon two important assumptions. First, that parasitised and unparasitised groups do not differ significantly in ways that might affect skew, other than the

differences in relatedness and RHP. Second, that subordinates on parasitised nests can detect the change in relatedness that occur upon replacement of the host dominant with the parasite. I explore each of these assumptions in turn below.

Whether an exploration of skew in parasitised colonies is useful for understanding processes determining skew in the host species crucially depends on the degree of similarity between parasitised and unparasitised colonies with respect to those factors likely to affect how reproduction is allocated. In *Polistes* co-foundress groups, the dominant is assumed to have either partial or total control over reproduction (Field & Cant 2009). This assumption is also likely to hold in parasitised colonies as the parasite rarely leaves the nest, is active in checking nest cells and interacts frequently with subordinates (Almond 2007; J. P. Green, personal observations). Additionally, there appears to be little difference in the behaviour of subordinates on parasitised and unparasitised nests, with subordinates engaging in similar rates of aggression (Almond 2007) and foraging activity (J. P. Green, unpublished data).

Potential differences may lie, however, in the benefits derived from group-living by subordinates and dominants in the two types of colony. In the Spanish *P. dominulus* population, Shreeves *et al*. (2003) have found evidence of assured fitness returns (AFRs), through which an individual's investment in brood care is preserved following its death through the continuing efforts of remaining group members. On parasitised nests, Shreeves *et al*. (2003) found that subordinate investment in the first brood is likewise preserved; however, with subsequent broods consisting largely of parasite offspring, there appears to be no scope for further AFRs, or indeed kin-selected benefits, for subordinates remaining on parasitised nests. However, the prospect of nest inheritance may provide an incentive for subordinates to remain on parasitised nests, in much the same way as it appears to favour the continuing presence of unrelated subordinates in unparasitised nests (Mead 1991; Leadbeater *et al*. 2011). Potential differences also occur in the benefits of group-living for host dominants and parasites. Whereas host dominants are able to nest alone, parasites appear completely dependent on the presence of hosts to rear their offspring (Cervo 2006). The threat of departure by subordinates is thus arguably stronger on parasitised nests, so that parasites might be expected to cede greater staying incentives in return for subordinate cooperation.

The second assumption underlying the approach taken in this study is that subordinates on parasitised nests are able to detect that the parasite is unrelated to them. As discussed above, there is currently no evidence that *P. dominulus* foundresses are able to respond to fine-scale intraspecific variation in relatedness, and instead appear to use natal nest cues as a guide to kinship. Whether foundresses are able to detect that a parasite on the nest is not a natal nest-mate is not completely clear; however, several lines of evidence suggest this may be the case. First, the violent and prolonged fighting that characterises the parasite's initial usurpation of the host nest (Zacchi *et al*. 1996; Chapter 6) would appear to provide hosts with information that they have been parasitised. Usurpation in many cases also triggers temporary abandonment of the nest by hosts (Zacchi *et al*. 1996; Chapter 6), which strongly suggests that hosts can detect the arrival of the parasite. It is, however, unclear whether hosts 'remember' this event in future interactions with their new dominant. While there is some evidence for (reasonably) robust visual memory in other *Polistes* species (Sheehan & Tibbetts 2008), there is no evidence for visual recognition by hosts of *P. semenowi* (J. P. Green, unpublished data; but see Ortolani *et al*. 2010). In addition, although chemical cues appear to facilitate host detection of the parasite at the point of invasion, subsequent changes in the parasite's odour profile to match that of the former dominant may interfere with the hosts' memory of the initial attack, and result in them accepting the parasite as a nest-mate (Lorenzi *et al*. 2004). The possibility therefore remains that hosts do not detect the presence of the parasite and do not adjust their estimate of relatedness to the dominant following parasitism, However, this seems somewhat unlikely, particularly given the high incidence of parasitism in some populations (e.g. Shreeves *et al*. 2003) and the fitness costs associated with parasite usurpation (see Chapter 1), which together would be expected to select for specific host responses to parasitism.

7.1.5 Aims

In this study, I aim to test the predictions of concessions and tug-of-war models by comparing reproductive skew between unparasitised *P. dominulus* colonies and colonies parasitised by the social parasite *P. semenowi* (see Table 7.1 below for a summary of the main predictions). To control for possible effects of other social and ecological factors on skew, parasitised and unparasitised nests were paired by group size,

productivity and site. Reproductive skew was examined in the first instance using microsatellites to determine the number of eggs laid by dominants and subordinates in each colony type. While molecular techniques allow the reproductive success of individuals to be accurately quantified, genetic data on their own may not be sufficient to illuminate the processes determining reproductive skew, and can give misleading estimates of skew if considered in isolation (Koenig *et al*. 2009). I therefore also investigated subordinate ovarian development and egg-laying behaviours in order to explore levels of reproductive investment by subordinates, as well as the opportunity for direct reproduction by subordinates, in parasitised and unparasitised colonies. As well as comparing skew between parasitised and unparasitised colonies, I also explored determinants of skew and ovarian development within each colony type to determine whether the sensitivity of reproductive partitioning to factors such as RHP and group size/productivity varies between parasitised and unparasitised colonies.

Table 7.1 Predictions made by skew models for parasitised and unparasitised colonies, based on differences in dominant-subordinate relatedness and RHP.

Colony type	Variable		Skew	
	Relatedness	RHP	Concessions	Tug-of-war
Parasitised	Lower	Higher	Lower	Higher
Unparasitised	Higher	Lower	Higher	Lower

7.2 Materials and Methods

7.2.1 Field methods

I located *P. dominulus* colonies at sites around Conil de la Frontera and Zahara de los Atunes (Cádiz Province, Spain) in April – May 2010 during the late pre-emergence phase of the colony cycle. Using intensive field surveys I identified 30 *P. dominulus* colonies parasitised by *P. semenowi*. I matched each parasitised colony with a nearby (< 0.5km) unparasitised colony of similar size, in terms of the number of adults on the nest (mean ± s.e. = 6.82 ± 0.42 adults, range: 3-20) and the size of the nest (73.1 ± 3.46 cells, range: 40-148). To stimulate egg-laying, I removed 10 eggs from each parasitised and unparasitised nest one week following initial colony identification. The delay of one week between nest identification and egg removal was to allow parasites that had usurped the nest only a short time before identification sufficient time to develop their ovaries. After a further 7 days, colonies were collected and nests and adults stored at -80°C at the University of Cádiz in preparation for genetic analysis in the UK.

7.2.2 Laboratory methods

All adults (parasites and hosts) collected with nests were genotyped at 9 microsatellite loci, together with 10 eggs chosen at random from each nest (where nests contained fewer than 10 eggs, all available eggs were genotyped). Laboratory procedures used to obtain the genotypes are described in detail in Chapter 2 (section 2.3). Due to the failure of DNA at locus Pdom127b to amplify, a maximum of 8 loci were available for use in assigning maternity. Tests for deviation from Hardy-Weinberg Equilibrium, linkage disequilibrium and heterozygote deficiency have previously been performed for the 8 microsatellites in a sample of *P. dominulus* females collected from the Spanish population in 2008-09 (see Leadbeater *et al*. 2010, 2011). In each case, no significant deviation from chance expectation was reported (Leadbeater *et al*. 2010, 2011).

I repeated these tests for the sample of 30 *P. semenowi* females used in this study. Observed and expected heterozygosities and estimated null allele frequencies were calculated using CERVUS v. 3.0 (Marshall *et al*. 1998; Kalinowski *et al*. 2007). Tests

for deviation from Hardy Weinberg Equilibrium and linkage disequilibrium were performed using GENEPOP v. 4 (Raymond & Rousset 1995). The results are presented in Appendix B. For all loci, the estimated frequency of null alleles was low (<0.05). Of the 8 loci, only one (Pdom140) showed significant heterozygote deficiency ($p = 0.007$), causing it to deviate from Hardy-Weinberg Equilibrium. In addition, two of the loci (Pdom25 and Pdom7) showed evidence of linkage disequilibrium ($p = 0.04$). However, deviation from Hardy-Weinberg and linkage equilibrium at these loci did not prevent accurate assignment of brood to adult parasites.

7.2.3 Maternity Assignment

Maternity assignment was performed using the software KINGROUP version 2.9 (Konovalov *et al*. 2004). I attempted to assign offspring to potential mothers where genotypes were available for at least 7 of the 8 loci. To assign maternity to offspring on parasitised nests, I first determined whether or not each offspring was produced by the parasite or one of the hosts by looking to see whether an offspring shared at least one allele at each locus with either the parasite or a host from the nest. This was made simpler by the presence of parasite-specific alleles at 2 loci, which were not present in any adult hosts. Parasite and host offspring were then separated. For both parasite and host offspring, I classed individuals that were homozygous at every locus as males, whereas I classed those that were heterozygous at one or more loci as females. Given the observed heterozygosities at the 8 loci, I calculated the probability of a *P. dominulus* female being homozygous at all 8 loci (and therefore of being incorrectly classed as a male) to be 1.01×10^{-6}. For a *P. semenowi* female, this probability was 7.24×10^{-5}. To assign maternity of host female offspring to individual hosts on a nest, I used the Full Sibship Reconstruction procedure (for details see Chapter 4, section 4.2.5), using the allele frequencies in the host population only (i.e. excluding parasite alleles). In doing so, I assumed that *P. dominulus* foundresses were singly-mated, following previous findings (Queller *et al*. 2000). Partitioning of parasite offspring into sibling groups is unnecessary as there is only one possible mother on the nest (i.e. one adult parasite).

I was able to assign all female offspring to individual adults on 49/60 nests. On a further three nests, the genotype data indicated reproduction by 'missing' wasps (i.e. the

offspring genotypes did not match the genotypes of any adult on the nest). In all cases, however, these missing wasps appeared to lay only a single egg and were not the dominant reproductives on the nests. I therefore included these nests in the analyses where possible, ignoring the contribution of missing wasps in calculations of skew and the proportion of offspring produced the dominant. On the remaining 8 nests, accurate assignment of female offspring to individual host mothers was not possible due to the existence of putative sisters with similar genotypes among the adults (one parasitised nest and 6 unparasitised nests) or else incomplete sequence data (one unparasitised nest). Despite difficulties in maternity assignment on these nests, I was nonetheless able to calculate skew for each nest. However, difficulties in determining the dominant individual in four of the unparasitised nests meant that it was not possible to look in detail at factors affecting skew on these nests.

Male offspring were present among the sample of eggs from 47/60 nests. On parasitised nests, I was unable to assign only 1/126 (0.8%) males to a particular adult on the nest; on unparasitised nests, however, this proportion was much higher (60%). Because of the bias in male offspring assignment between parasitised and unparasitised colonies, I decided to omit male offspring when estimating skew. The analyses of reproductive skew presented that follow thus refer to skew in production of female offspring only.

7.2.4 Genetic relatedness

For each unparasitised nest, I estimated the average relatedness between a dominant and each of its subordinates using the software RELATEDNESS version 5.0.8 (Queller & Goodnight 1989). Nests were weighted equally and standard errors obtained by jack-knifing over nests. Dominant-subordinate relatedness was estimated for subordinates on 26 unparasitised nests. Relatedness could not be estimated for four nests where the identity of the dominant could not be determined.

7.2.5 Measuring reproductive skew

Skew within parasitised and unparasitised nests was calculated in two ways. First, I calculated the B (binomial) index (Nonacs 2003a), which measures variance in

reproduction among group members relative to the variance expected if all individuals have an equal opportunity of reproducing. Values of B can range from -1 to 2; a value of 0 indicates that reproduction is randomly distributed among nest-mates, whereas positive values indicate a greater skew in reproduction than expected by chance and negative values indicate a more even distribution than expected by chance (Nonacs 2003a). Values of the B index and significance tests were calculated using the software Skew Calculator 2.1 (Nonacs 2003b). Second, I calculated the proportion of eggs laid by the dominant on each nest (where the dominant was defined as the individual in the group that laid the greatest number of eggs). In addition to calculating overall skew within nests, I also calculated the proportion of eggs in each dominant-subordinate pair that was produced by the dominant, which I denote P_d. P_d was calculated for all nests where a dominant could be identified from the sequence data (n = 56 nests).

The number of potential reproductives on a nest was estimated as the number of individuals present at nest collection (plus any missing wasps, where their presence was indicated by offspring genotypes). On seven nests, first-generation female offspring (workers) were present at nest collection. Although capable of reproducing, workers were not included as potential reproductives when calculating skew for two reasons. First, the absence of adult males in the population at the time of nest collection meant that emerging workers did not have the opportunity to mate. Second, while unmated workers are still able to lay male eggs, dissections revealed no ovarian development among workers, ruling them out as potential mothers of the offspring I genotyped.

7.2.6 Morphological measurements

As noted above, studies of skew in primitively eusocial wasps have often equated RHP with body size, despite a striking lack of data in support of this assumption. Unusually, however, in *P. dominulus* there is good evidence that body size predicts RHP, both in intraspecific (Tibbetts & Shorter 2009) and interspecific contests (Ortolani & Cervo 2010; Cini *et al*. 2011; Green & Field 2011a; Chapter 6). RHP was therefore assessed using body size, measured as the width of the head. For each wasp, I removed the head and placed it on a microscope slide. Head width was measured at the widest point of the head using a 16× binocular microscope and graticule. For each subordinate, I calculated

its size relative to the dominant as the difference in size between dominant and subordinate, divided by the mean for the pair. For each nest, I calculated the difference in size between a dominant and the mean size of subordinates on the nest. This value was then divided by the mean size of all group members in order to obtain a standardised difference that could be compared across nests.

As a second measure of RHP, I recorded the presence/absence of subordinate clypeal patterning. Clypeal patterning (in particular, the disruption or brokenness of the pattern) has been argued to function as a signal of RHP in *P. dominulus* (e.g. Tibbetts & Dale 2004; Tibbetts & Lindsay 2008). However, this finding has been repeatedly questioned in European populations, where, in particular, there is little evidence that pattern brokenness signals RHP (Cervo *et al*. 2008; Green & Field 2011b; Chapters 3 & 4). Nonetheless, I include pattern brokenness as a potential indicator of subordinate RHP in this study based on the curious finding in the previous chapter that usurpation fights between hosts and invading parasites (*P. semenowi*) were longer in contests where a greater proportion of defending hosts had clypeal patterns, suggesting that clypeal patterns may reflect RHP during fights over nest ownership (Green & Field 2011a; Chapter 6). The presence/absence of clypeal patterns was recorded, as this is argued to be a simple proxy for pattern brokenness (Tibbetts & Lindsay 2008): hosts without black spots have a lower brokenness (i.e. 0) than hosts with spots. Head size and clypeal pattern data were collected for individuals on 27 parasitised and 24 unparasitised nests.

Ovarian development (OD) was assessed by performing dissections of ovaries in 10% saline under a 40× dissecting microscope. Polistine wasps possess two ovaries, each comprising three ovarioles (Spradberry 1973). Following previous studies (e.g. Cant *et al*. 2006), OD was quantified by recording the length of the largest egg in each ovariole using a graticule (analyses of OD in which egg size was substituted for the mean number of eggs in each ovariole did not alter the results). I also noted the presence of yellow bodies (corpora lutea) at the base of the ovaries. Yellow bodies constitute the remains of nurse cells after eggs have been laid, and have been used in previous studies as an indication of recent oviposition (e.g. Liebert & Starks 2006). OD data were collected for 246 individuals (202 subordinates and 44 dominants) from 24 parasitised and 20 unparasitised nests.

7.2.7 Observations of subordinate egg-laying

Subordinate egg-laying was examined by analysing video footage of subordinate activity from a previous study (Almond 2007). Footage of subordinate egg-laying was recorded on 15 parasitised and 17 unparasitised nests during the pre-emergence phase of the colony cycle in 2004 and 2005 at several of the field sites used in this study. Subordinates were identified using behavioural censuses (see Chapter 4). For each nest, the frequency of egg-laying attempts by subordinates was recorded over a one hour period between 1100 and 1330. I distinguished between successful laying attempts and unsuccessful attempts where another individual inspected the cell within 30s of the subordinate laying and appeared to engage in oophagy. On all nests, dominants had been marked with individual paint spots to aid identification. Subordinates were not marked, however, meaning that it was not possible to distinguish between multiple egg-laying attempts by the same subordinate or separate attempts by different subordinates.

7.2.8 Statistical analyses

Reproductive Skew

I compared skew using both the B index and the proportion of eggs laid by the dominant between matched pairs of parasitised and unparasitised nests using Wilcoxon signed rank tests. On three parasitised nests, only one female offspring was produced. Because B is undefined for groups containing one offspring, these nests (and their matching unparasitised nests) were omitted from the analysis. Values of B were thus compared between 27 pairs of parasitised and unparasitised nests, while the proportion of dominant-laid eggs was compared between all 30 pairs of nests.

To explore determinants of skew among nests in more detail, I chose to focus on the proportion of eggs produced by the dominant on a nest, as this provides a more straightforward indication of the extent to which dominants monopolise reproduction than does the B index. I used GLMs fitted with either binomial or quasibinomial errors (where there was evidence of overdispersion in the data). In the first analysis, I compared the proportion of dominant-laid eggs between parasitised and unparasitised

nests by fitting dominant ID (host or parasite) as an explanatory variable. Group size, productivity (estimated as the number of nest cells) and the presence of workers were also fitted as explanatory variables, together with all first-order interactions. The presence of workers was included as a predictor of skew because there is some suggestion from studies of other *Polistes* species that the emergence of workers precipitates important changes within the colony, including the possible eviction of subordinates by the dominant (Reeve 1991), as well as increased rates of dominant turn-over (Leadbeater *et al*. 2011), both of which may affect reproductive partitioning.

The proportion of dominant-laid eggs was then examined separately on parasitised and unparasitised nests in two further GLMs. In both, I fitted the following explanatory variables: average dominant-subordinate size difference, the proportion of subordinates with clypeal patterns, group size and productivity, and the presence of workers. For the analysis of unparasitised nests, I also fitted average dominant-subordinate relatedness as an additional explanatory variable. Overall, I was able to collect data on size and clypeal patterning for individuals on 26 of 30 parasitised nests and data on size, patterning and relatedness for individuals on 24 of 30 unparasitised nests. Thus, in the above analyses, n = 26 parasitised and 24 unparasitised nests.

Ovarian development

To check the reliability of the maternity assignment procedure, I compared OD between dominant and subordinate nest-mates (n = 24 parasitised nests and 20 unparasitised nests), using Wilcoxon signed-rank tests. To test whether subordinate OD varied between parasitised and unparasitised nests, I compared mean subordinate OD on 19 pairs of parasitised and unparasitised nests (paired by site, group size and productivity), again using Wilcoxon signed-rank tests.

Within groups, a subordinate's OD is unlikely to be independent from that of its nest-mates, for two reasons. First, levels of OD for all subordinates within a group are determined (to an extent) through interactions with a common dominant. Second, the level of OD exhibited by a particular subordinate is likely to be partly determined by the levels of OD of other subordinates in a group. This may be for a number of reasons,

including limited opportunities for direct reproduction (i.e. reproduction is a zero-sum game) or else suppression of OD in some group members by others with more developed ovaries (Röseler 1991). To look in more detail at factors affecting subordinate OD, I therefore used a combination of randomisation tests and GLM (see Chapter 4). Separate analyses were carried out for parasitised and unparasitised colonies. Randomisation tests were used to test for correlations between OD and dominant-subordinate size difference and relatedness. I began by comparing the mean within-nest correlation between OD and dominant-subordinate size differences with that obtained using simulated groups in which OD was randomised among subordinates. First, I calculated Spearman's rank correlation (ρ) between OD and size differences for each group, and then calculated the overall observed mean correlation across groups. I then recalculated ρ after OD was randomly permuted in each group, to obtain an overall simulated mean correlation. I repeated this simulation procedure 10 000 times to obtain a null distribution of means. The proportion of simulated means equal to or stronger than the observed mean was used as an estimate of the probability (p) of obtaining the observed correlation by chance. Correlations were considered to be significant if $p < 0.05$. This procedure was repeated for parasitised and unparasitised nests. The same procedure was also used to test the relationship between subordinate OD and relatedness on unparasitised nests.

Because each explanatory variable is examined separately, the simulations do not control for any correlations between explanatory variables or for effects of other factors such as group size and productivity. I therefore analysed the combined effect of these variables on subordinate OD in a GLM with Tweedie errors, which are suitable for continuous data with many zero values (Dunn & Smyth 2004). Because levels of subordinate OD in a group are not independent, I sampled one subordinate at random from each group. Subordinate OD was the response variable and dominant-subordinate size difference, group size and group productivity were fitted as explanatory variables. For the analysis of subordinate OD on unparasitised nests, relatedness to the dominant was added as an additional explanatory variable. I repeated the procedure of sampling followed by GLM 2000 times in order to determine how frequently significant results were obtained at the 95% confidence interval. In the absence of an effect of the variable of interest, a significant result would still be expected in 5% cases. I considered

explanatory terms to be significant if their associated *p* values < 0.05 in at least 5% of the re-sampling runs. Due to the low frequency of subordinates with clypeal patterns, it was not possible to test the effect of clypeal patterning on OD using the analysis described above. I therefore used Mann-Whitney U tests to compare OD of subordinates with and without clypeal patterns across all nests and within parasitised and unparasitised nests.

Subordinate egg-laying

The frequency of subordinate egg-laying was compared between parasitised and unparasitised nests using a binomial test. The proportion of egg-laying attempts that were successful was compared between parasitised and unparasitised nests using Fisher's exact test.

Sample sizes

For clarity, the number of nests used in each analysis is given in Table 7.2 (overleaf).

Table 7.2 Numbers of nests used in analyses.

Analysis	**Number of nests**
Reproductive skew	
Skew (B) (parasitised vs. unparasitised)	27 vs. 27
% dominant-laid eggs (parasitised vs. unparasitised)	30 vs. 30
% dominant-laid eggs in parasitised nests	26
% dominant-laid eggs in unparasitised nests	24
Ovarian development	
Subordinate OD (parasitised vs. unparasitised)	19 vs. 19
Subordinate OD in parasitised nests	24
Subordinate OD in unparasitised nests	20
Egg-laying	
Subordinate egg-laying (parasitised vs. unparasitised)	15 vs. 17

7.3 Results

7.3.1 Characteristics of parasitised and unparasitised nests

Comparing skew between parasitised and unparasitised colonies offers an opportunity to test the predictions of competing skew models due to the differences in relatedness and RHP between dominants and subordinates. The relatedness between a parasite and its subordinates on parasitised nests is zero, while on unparasitised nests, the average

relatedness between a dominant foundress and her subordinates ranged from -0.02 to 0.91, with a mean relatedness of 0.60 ± 0.02. On 22 of 27 parasitised nests, the parasite was the largest individual, while the dominant was the largest foundress on only 9 of 24 unparasitised nests. If dominance were random with respect to size, the dominant would be expected to be the largest foundress on 5/24 nests. This is not significantly different to the observed proportion (Exact binomial test, $p = 0.07$), indicating that size does not predict dominance among host foundresses. Importantly, the size difference between dominants and subordinates was significantly greater on parasitised nests than on unparasitised nests (Welch's t-test: $t_{40} = 5.00$, $p < 0.0001$). The expected differences between parasitised and unparasitised colonies in relatedness and RHP were therefore observed: subordinates were on average more closely related and more similar in size to dominants on unparasitised nests than to parasites on parasitised nests.

7.3.2 Reproductive skew

Skew on parasitised vs. unparasitised nests

Despite the differences in relatedness and RHP, skew in both parasitised and unparasitised groups was high. The mean value of B on parasitised nests was 0.57 ± 0.04. On 22 of 27 parasitised nests, values of B were significant at $p < 0.05$, indicating that the distribution of reproduction on these nests was more skewed than expected under a random distribution of reproduction among group members. Across all unparasitised nests, the mean value of B was 0.54 ± 0.04, and values of B were significant at $p < 0.05$ for 24 of 30 nests. When accounting for multiple tests using sequential Bonferroni adjustment, values of B were significant at $p < 0.05$ for 15/27 parasitised and 23/30 unparasitised nests. Values of B did not differ significantly between parasitised and unparasitised nests ($Z = 0.09$, $n = 54$, $p = 0.94$; Figure 7.2a). Similar patterns are seen in the proportion of dominant-laid eggs on parasitised and unparasitised nests. Among parasitised nests, the mean (un-weighted) percentage of eggs laid by the parasite was 97 ± 0.02%. On 26 of 30 nests, the parasite was the only individual to produce offspring; on the remaining four nests, a single offspring was produced by a host subordinate. Among unparasitised nests, the mean (un-weighted) percentage of dominant-laid eggs was 90 ± 0.03%. On 21 of 30 nests, the dominant was

the only individual to produce offspring. On a further 5 nests, a single subordinate produced offspring, while on three nests two subordinates produced offspring and on one nest three subordinates produced offspring. Although the percentage of eggs laid by the dominant was higher on parasitised nests than on unparasitised nests (97 vs. 90%), this difference was not statistically significant (Z = 1.73, n = 60, p = 0.09; Figure 7.2b).

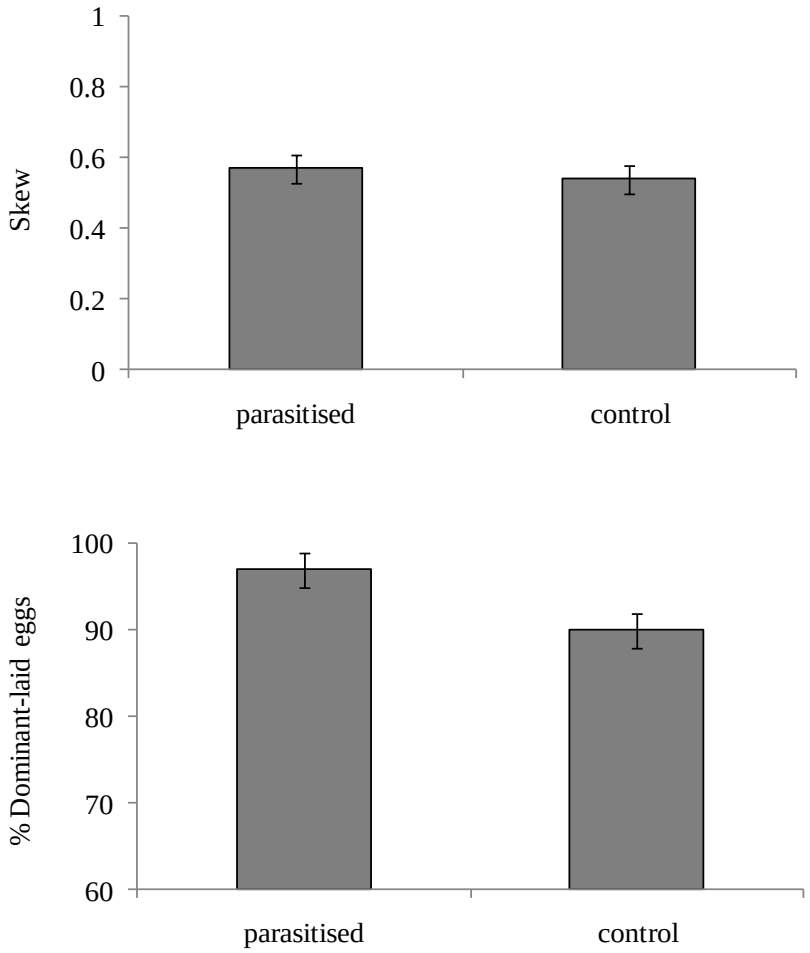

Figure 7.2 Reproductive skew in parasitised and unparasitised colonies. (a) B values for 27 parasitised and 27 unparasitised colonies. (b) Percentage of offspring produced by the dominant on 30 parasitised and 30 unparasitised nests. Means are shown ± 1 s.e.

On one unparasitised nest where low skew was observed (CC4), the partitioning of offspring into multiple sibling groups was based on genotypes from only three loci.

Excluding this nest and the matching parasitised nest from the above analyses did not affect the result. On two further unparasitised nests (M1 and PS11), offspring that were assigned to two mothers (the dominant and a subordinate) could have been produced by a single individual (i.e. the dominant) that had mated multiply. In this study, I have assumed single mating based on the results of previous research (Queller *et al*. 2000). However, if the dominant was multiply mated on these nests, reproductive skew would in reality be higher than is currently supposed (50% vs. 88% dominant offspring and 50% vs. 90% for single vs. multiple mating in M1 and PS11 respectively).

Results of the above analysis showed that the proportion of dominant-laid eggs, while higher on parasitised nests, did not differ significantly between parasitised and unparasitised nests. However, the results of a more powerful analysis of the data using GLM showed that the difference in the proportion of dominant-laid eggs between nest groups was statistically significant ($F_{1,58}$ = 4.83, p = 0.03). However, this result was strongly dependent on one unparasitised nest (CC4), in which the dominant produced only 56% offspring. As mentioned above, the division of offspring on CC4 into multiple sibling groups was based on genotypes at only three loci. When this nest was excluded, the difference in skew between parasitised and unparasitised nests was not significant ($F_{1,57}$ = 3.51, p = 0.07). Across all nests (including CC4), the GLM analysis also showed that the proportion of offspring produced by the dominant was not significantly predicted by group size ($F_{1,57}$ = 2.39, p = 0.13) or productivity ($F_{1,57}$ = 0.43, p = 0.51).

Determinants of skew on parasitised and unparasitised nests

On parasitised nests, the proportion of eggs laid by the parasite was not predicted by its size relative to its subordinates (χ^2_1 = 0.15, p = 0.70) or by the proportion of subordinates with clypeal patterns (χ^2_1 = 0.11, p = 0.73). The proportion of parasite-laid eggs was also unaffected by group size (χ^2_1 = 0.64, p = 0.42), productivity (χ^2_1 = 0.06, p = 0.81), or the presence of workers (χ^2_1 = 1.11, p = 0.29). Similar results were obtained for unparasitised nests. The proportion of eggs laid by the dominant was not predicted by its size relative to its subordinates ($F_{1,57}$ = 0.44, p = 0.52) or by the proportion of subordinates with clypeal patterns ($F_{1,22}$ = 0.10, p = 0.76). There was also no relationship between the proportion of dominant-laid eggs and the average relatedness

between a dominant and its subordinates ($F_{1,22} = 1.65$, $p = 0.21$). As on parasitised nests, the proportion of dominant-laid eggs was also unaffected by group size ($F_{1,22} = 1.64$, $p = 0.21$), productivity ($F_{1,22} = 0.06$, $p = 0.80$), or the presence of workers ($F_{1,22} = 1.31$, $p = 0.27$). Excluding nest CC4 did not alter the results.

In addition to calculating the proportion of eggs laid by the dominant on each nest, I calculated the proportion of eggs laid by the dominant for each dominant-subordinate pair (P_d) on 30 parasitised nests and 24 unparasitised nests. Among all 209 pairs, $P_d < 1$ in only 12 pairs (4 pairs on parasitised nests and 8 pairs on unparasitised nests). In the other 197 pairs, $P_d = 1$. The small proportion of pairs with values of $P_d < 1$ meant that it was not possible to test the effects of potential variables using statistical techniques that account for the non-independence in the data. However, as an inspection of the data makes clear, there is no sign of any relationship between P_d and the size of dominants relative to their subordinates, either in parasitised or unparasitised colonies (Figure 7.3). There is also no sign of a relationship between P_d and the average relatedness between a dominant and its subordinate in unparasitised colonies (Figure 7.4).

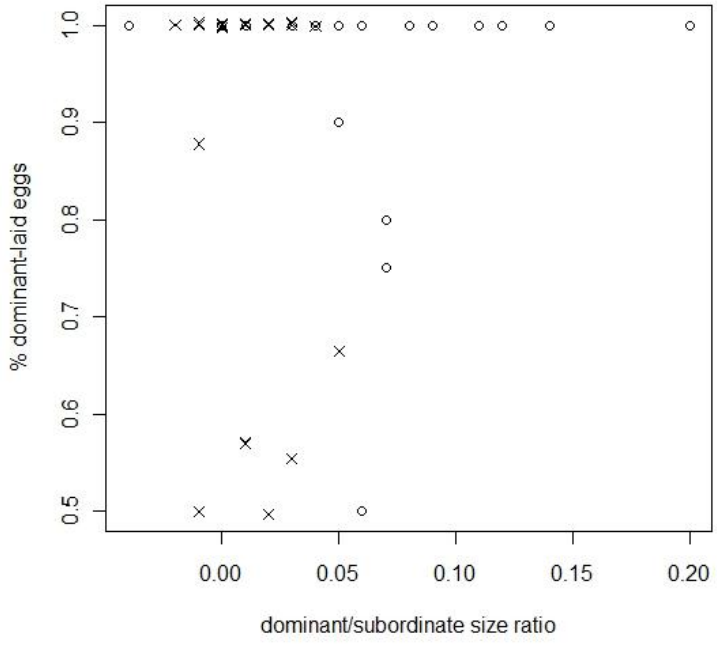

Figure 7.3 Proportion of dominant-laid eggs and dominant-subordinate size ratios in parasitised (o) and unparasitised (×) nests. Points are jittered to show overlapping data.

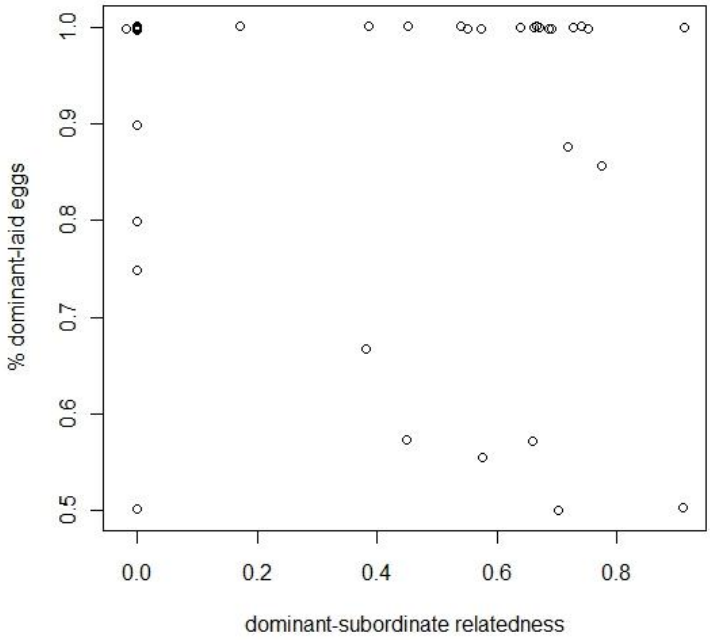

Figure 7.4 Proportion of dominant-laid eggs and average dominant-subordinate relatedness on unparasitised nests. Points are jittered to show overlapping data.

7.3.3 Ovarian development

OD data were in broad agreement with the maternity assignment based on the genotype data: within both parasitised and unparasitised nests, individuals identified as dominant using the sequence data had greater OD than those identified as subordinate (parasitised nests: $Z = -4.23$, $n = 24$, $p < 0.0001$; unparasitised nests: $Z = -3.06$, $n = 20$, $p = 0.001$). Subordinate OD was nonetheless substantial, with 76% subordinates possessing at least one clearly-defined egg in one or more ovarioles. Among subordinates that failed to produce offspring, 75% showed some degree of OD. However, data for five nests on which subordinates reproduced showed that those with offspring had significantly greater OD than those without offspring (24 ± 3.17 vs. 8.51 ± 2.61μm; 1-tailed Wilcoxon-signed rank test, $Z = 2.02$, $n = 5$, $p = 0.03$), indicating that reproductive success among subordinates depends on OD. A comparison of mean subordinate OD on parasitised and unparasitised nests showed that subordinate OD was slightly higher on parasitised nests, though this difference was not significant (egg size: 14.39 ± 1.60 vs.

12.96 ± 2.06μm, Z = -1.39, n = 19 pairs of nests matched for site, group size and productivity, p = 0.17).

Factors affecting subordinate OD were examined in more detail using randomisation tests and GLM. On parasitised nests, simulations revealed a positive correlation between a subordinate's OD and its size relative to the dominant, though this correlation was not quite significant (mean ρ = 0.25, p = 0.06; Figure 7.5). Similar results were obtained in the GLM (mean t = 1.00, $p < 0.05$ for 4.9% simulations). On unparasitised nests, simulations again revealed a positive correlation between OD and size relative to the dominant, which in this case was marginally significant (mean ρ = 0.22, p = 0.05). However, when analysed in the presence of other predictors, this correlation was no longer significant (mean t = 0.97, $p < 0.05$ for 3.15% simulations). There was no correlation between OD and relatedness to the dominant on unparasitised nests, either in the simulations (mean ρ = -0.21, p = 0.12) or in the GLM (mean t = 0.62, $p < 0.05$ for <1% simulations). On unparasitised nests, subordinate OD was significantly predicted by group size, with subordinates showing reduced OD in larger groups (mean t = 1.45, $p < 0.05$ for 20.7% simulations). However, there was no relationship between OD and productivity (mean t = 0.69, $p < 0.05$ for 1.65% simulations). On parasitised nests, subordinate OD was also significantly negatively related to group size (mean t = 0.82, $p < 0.05$ for 6.05% simulations). Subordinate OD was also significantly related to productivity, with greater OD on more productive nests (mean t = 1.22, $p < 0.05$ for 13.9% simulations). Finally, across both unparasitised and parasitised nests, subordinate OD was not predicted by the presence of clypeal patterns (Z = -0.45, n = 20, p = 0.67). Within groups, clypeal patterning did not predict subordinate OD either on unparasitised nests (Z = 0.86, n = 12, p = 0.42) or on parasitised nests (Z = -0.70, n = 8, p = 0.55).

Previous studies have used the presence of yellow bodies as indicators of recent oviposition in *Polistes* (e.g. Liebert & Starks 2006). In this study, however, the presence of yellow bodies did not accurately predict patterns of reproduction as determined by the molecular data. In particular, yellow bodies were never observed in the ovaries of parasites, which were the dominant reproductives on all but one of the parasitised nests.

On unparasitised nests, yellow bodies were found in only 4 of 24 dominant foundresses, and their presence did not reliably predict reproduction among subordinates.

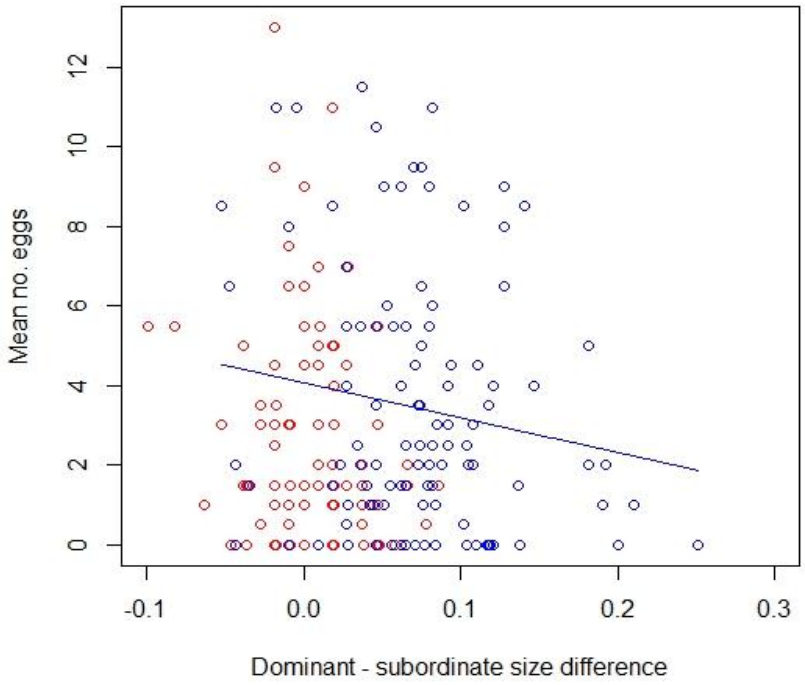

Figure 7.5 Relationship between subordinate egg number and dominant-subordinate size difference in parasitised (red) and unparasitised (blue) colonies. The best-fit line was generated using a GLM with Tweedie errors with size difference as the explanatory variable.

7.3.4 Subordinate egg-laying

Video analysis identified 13 instances of subordinate egg-laying on four parasitised nests and two unparasitised nests. The frequency of subordinate egg-laying on parasitised nests was higher than on unparasitised nests (9 vs. 4 attempts), though this difference was not significant (binomial test, $p = 0.27$). On parasitised nests, 2 of the 9 egg-laying attempts recorded were successful, while 7 were unsuccessful (i.e. were immediately followed by cell inspection by a second wasp, in all cases the parasite). On unparasitised nests, 3 of the 4 egg-laying attempts recorded were successful and one was unsuccessful (in the latter case, it was not possible to tell whether the cell was

inspected by the dominant or another subordinate after the initial egg-laying event). Overall, the proportion of unsuccessful attempts was higher on parasitised nests than unparasitised nests, though again this difference was not statistically significant (Fisher's exact test, $p = 0.22$).

7.4 Discussion

Reproductive skew models have come under increasing criticism in recent years for the great number and complexity of their predictions (Heinsohn & Magrath 2000; Koenig *et al*. 2009; Taborsky 2009). In particular, attempts to distinguish between different modes of reproductive partitioning have been complicated by the fact that different models may sometimes make the same predictions about the relationship between skew and one or more social or ecological variables (Heinsohn & Magrath 2000; Koenig *et al*. 2009). Therefore, a number of authors have argued that, rather than attempting to find a particular model that fits the empirical data, tests of skew theory should focus on comparing the predictions of competing models, ideally using experimental manipulations, and taking care to select models with realistic assumptions (Magrath & Heinsohn 2000; Kokko 2003; Field & Cant 2009; Koenig *et al*. 2009; Taborsky 2009). The aim of this study was to use social parasitism to test the predictions of concessions and tug-of-war models in the paper wasp *P. dominulus* by comparing skew in parasitised and unparasitised colonies using a large sample size (60 nests). If the dominant has total control of reproduction and cedes reproduction to subordinates in order to retain them peacefully in the group, as assumed by concessions models, lower skew is expected on parasitised nests due to the low relatedness between the parasite and its subordinates (see Introduction). However, if the dominant has only partial control over reproduction, and skew is determined via competition between group members, as assumed by tug-of-war models, higher skew is expected on parasitised nests due to the greater asymmetry in RHP between the parasite and its subordinates.

Overall, the results of this study found no support for concessions models. Across all nests, reproductive skew was generally high, consistent with levels reported in previous studies of *P. dominulus* (Queller *et al*. 2000; Liebert & Starks 2006; Leadbeater *et al*.

2011). However, skew was slightly higher on parasitised nests, which is the opposite pattern to that predicted if subordinates receive reproductive incentives from the dominant. In particular, this result suggests that subordinates who are more distantly related to the dominant do not receive greater direct benefits to compensate for reduced kin-selected benefits available through helping, a finding that is again consistent with previous work (Queller *et al*. 2000; Liebert & Starks 2006; Leadbeater *et al*. 2011).

Although the high level of skew on parasitised nests appears to refute the predictions of basic concessions models, can this result be reconciled with more recent extensions of transactional skew theory? In their dynamic concessions model, Kokko & Johnstone (1999) showed that the opportunity for inheritance of the dominant position can dramatically reduce the staying incentive required by subordinates. Indeed, the fitness pay-off from inheriting the dominant position can be great enough to retain unrelated subordinates in the absence of any reproductive concessions (Kokko & Johnstone 1999). Could the prospect of future reproduction therefore explain the high skew observed, particularly on parasitised nests where relatedness is low? In a recent study, Leadbeater *et al*. (2011) showed that the frequency of inheritance within unparasitised *P. dominulus* groups was sufficient to favour nest joining and subordination over independent nesting, in spite of the high reproductive skew in co-foundress groups. Indeed, the fitness pay-offs associated with nest inheritance were found to be great enough to favour joining by unrelated individuals (Leadbeater *et al*. 2011), which appears to support the prediction of Kokko & Johnstone's (1999) model. While the frequency of nest inheritance in parasitised groups remains to be quantified, there is anecdotal evidence that host subordinates can inherit the nest following the death or disappearance of the parasite (Mead 1991), which suggests that nest inheritance may act to limit the concessions required by subordinates to remain within parasitised groups.

High skew on parasitised nests may also be consistent with reproductive concessions if parasitised colonies are saturated with subordinates (Reeve & Emlen 2000; Reeve & Keller 2001). Extending the basic concessions model to consider skew in groups of varying sizes, Reeve & Emlen (2000) show that the size of concessions offered to subordinates can vary independently of relatedness in groups that are saturated with subordinates (i.e. where further joining is not favoured by the dominant). Reeve &

Emlen (2000) argue that groups are more likely to be saturated when constraints on independent breeding are low and relatedness is high. Given that reproductive constraints in parasitised colonies are likely to be high and that relatedness is low, it is unlikely that parasitised colonies are saturated, meaning that larger concessions should be offered by the parasite in response to low intra-group relatedness, as predicted by traditional concessions models.

Ultimately, however, neither the dynamic concessions model nor the N-person concessions model can successfully account for the observed patterns of reproduction on parasitised and unparasitised nests. This is because, in both models, as well as in the basic concessions model, skew is assumed to be the result of an 'agreement' between the dominant and subordinate over the share of reproduction that each receives, given the benefits to each of maintaining the association (Johnstone 2000). Consequently, the reproductive share that a subordinate receives should be reflected in its level of reproductive investment, assuming that this investment incurs a cost. Although it was shown that subordinates that successfully reproduced had greater OD than those that failed to reproduce, nonetheless non-reproductive subordinates were found to have significant levels of ovarian development. This latter finding, together with evidence from observations of egg-eating following subordinate oviposition attempts, indicates that a subordinate's reproductive share is unlikely to be determined by a negotiated concession from the dominant, but rather appears to be the result of on-going competition between group members over reproduction.

In general, transactional models of skew have received very limited support in *Polistes*, both in *P. dominulus* (Queller *et al*. 2000; Liebert & Starks 2006) and in other species (Field *et al*. 1998; Seppä *et al*. 2002; Liebert *et al*. 2005; Nonacs *et al*. 2006; reviewed in Nonacs 2006; Field & Cant 2009). Strong evidence for reproductive transactions comes from only a single study, that by Reeve *et al*. (2000), which found that skew in *P. fuscatus* groups was positively correlated with relatedness and group productivity. Reeve *et al*. (2000) also found that skew among late offspring was higher than among early offspring. This finding was interpreted as evidence of a staying incentive, the size of which decreased over the season in response to an increase in the constraints on independent breeding. However, Nonacs (2006) and Field & Cant (2009) have since

argued that increasing skew could be the result of a decline in subordinate RHP over the season, allowing the dominant to wrest a larger share of reproduction in a tug-of-war as the season progresses. In general, the failure of transactional skew models to predict skew in *Polistes* and other social taxa may be due, in part, to unrealistic assumptions about how reproduction is controlled, as well as the ability of individuals to gather and act on information about the social and ecological factors that determine the relative benefits of cooperating versus nesting alone, an issue I address in greater detail in the following chapter (Clutton-Brock 1998; Kokko 2003; Field & Cant 2009).

If concessions models are unable to explain the patterns of reproductive skew and ovarian development in parasitised and unparasitised colonies, do such patterns instead support a tug-of-war over reproduction based on differences in power between group members? Several lines of evidence suggest that reproduction may be determined through competition. Firstly, the higher levels of skew in parasitised colonies compared with those in unparasitised colonies are consistent with a tug-of-war over reproduction, which is won more frequently by the parasite than the host dominant due to the parasite's superior RHP compared to that of its subordinates. However, it is important to note that the difference in skew between parasitised and unparasitised colonies was not large (97% vs. 90% dominant-laid eggs) and depended strongly upon one unparasitised colony where maternity assignment was based on incomplete genetic data. Secondly, patterns of subordinate OD are also reasonably consistent with a tug-of-war over reproduction, in particular the finding that many subordinates invest in reproduction (i.e. developed ovaries), despite apparently producing no offspring. As noted above, this finding indicates that subordinates are likely to be attempting to obtain reproduction that is not sanctioned by the dominant, as evidenced by the observations of possible oophagy following subordinate oviposition.

However, support for the central prediction of tug-of-war models – that skew should decrease with increasing subordinate RHP – is more limited. Although skew was higher in parasitised colonies, where asymmetries in dominant-subordinate RHP are expected to be large, the size of a subordinate relative to the dominant had no effect on the amount of reproduction it received in either parasitised or unparasitised colonies. This might suggest that either skew is higher in parasitised colonies for reasons other than

differences in dominant-subordinate RHP or else some aspect of RHP other than body size is important in determining a subordinate's share of reproduction. Clypeal patterns have been argued to provide a reliable indication of RHP in *P. dominulus*, based on observations of dominance interactions and usurpation contests in the lab (Tibbetts & Dale 2004; Tibbetts & Shorter 2009; Green & Field 2011a; Chapter 6). However, in this study, clypeal patterning did not predict a subordinate's share of reproduction. Furthermore, the proportion of subordinates with clypeal patterns did not predict skew in either colony type, indicating that a subordinate's scope for reproduction within a group is not determined by any potential traits associated with clypeal patterning (see Chapter 4 for discussion of a similar result).

While body size does not predict the amount of reproduction obtained by subordinates, there is nonetheless some indication that size may be important in determining a subordinate's scope for reproduction. Subordinates that were smaller in size relative to their dominant tended to have reduced OD, though this effect was small, and was not significant in some analyses. One explanation is that low levels of OD are simply a consequence of small body size; however, previous work suggests that size does not predict OD in *P. dominulus* (Röseler 1991; but see Reeve 1991). Alternatively, smaller subordinates may be more vulnerable to efforts by the dominant to suppress OD. If this were the case, however, then levels of OD should be lower among subordinates in parasitised colonies than in unparasitised colonies on account of the greater size difference between subordinates and parasites, yet no significant difference was found. Reproductive suppression also appears inconsistent with the observed relationship between subordinate reproduction and group size. If skew were the result of dominant suppression of subordinate reproduction, skew might be expected to be higher in smaller groups, assuming that suppression of subordinate reproduction is more effective in small groups. However, this was not the case. There was no relationship between skew and group size, and subordinate OD actually decreased with increasing group size both in parasitised and unparasitised colonies. Therefore, while larger subordinates possess more developed ovaries, it is not clear whether this is a consequence of larger size conferring a competitive advantage in a tug-of-war of reproduction.

Irrespective, however, of the possible influence of RHP on the level of subordinate reproductive investment, the absence of a relationship between a subordinate's RHP and its realised reproductive success (i.e. the number of offspring it succeeds in producing) would seem to present a difficulty for the tug-of-war model. That skew within unparasitised groups spanning a range of dominant-subordinate size differences was found to be consistently high appears to argue against reproductive partitioning based on competition. Moreover, this result is unlikely to be due to the focus of this study on body size as a measure of RHP as, unusually in *Polistes*, there is good evidence that size affects the outcome of competitive interactions in *P. dominulus* (Tibbetts & Shorter 2009; Cini *et al*. 2011; Green & Field 2011a, Chapter 6). However, it is possible that competition occurs principally through an effect on reproductive investment, explaining why smaller subordinates were found to have reduced OD, and that this is reinforced by dominant behaviours such as differential oophagy, thereby maintaining high skew.

In general, however, high levels of skew across a range of social and ecological variables appear inconsistent with transactional and tug-of-war models of reproductive skew (Field & Cant 2009). Could the patterns of reproduction seen in *P. dominulus* instead be explained by the use of a convention (that is, respect for an arbitrary cue) to determine access to reproduction (Field & Cant 2009)? The use of conventions has been argued to reduce the risk (and need) for escalated conflict between individuals, and in doing so stabilise cooperation and boost group productivity (Field & Cant 2009). In several primitively eusocial wasp species, conventions appear to dictate the allocation of reproduction in cooperating groups – for example, the age-based dominance hierarchy (gerontocracy) in the hover wasp *L. flavolineata* (Bridge & Field 2007) and a possible convention based on order of arrival at the nest in *P. carolina* (Seppä *et al*. 2002). In the latter species, however, continued aggression among foundresses during the nest-founding phase suggests that this convention is not adhered to absolutely, and that individuals may still try to secure reproduction through competition (Seppä *et al*. 2002). High levels of aggression also characterise nest founding in *P. dominulus*, which has led previous authors to assume that dominance hierarchies in this species principally reflect asymmetries in RHP between rival co-foundresses (e.g. Pardi 1948; Tibbetts & Dale 2004). Indeed, recent efforts to uncover a peaceful convention in this species have met with little success (Zanette & Field 2009). In this study, while the high levels of

skew across groups are consistent with conventional settlements, support for conventions is undermined by the substantial levels of ovarian development among subordinates. As noted above, the presence of many subordinates with well-developed ovaries suggests active competition over reproduction, rather than a respect for reproductive allocation based either on transactions or conventions.

In conclusion, the results of this study reject a mode of reproductive partitioning based on dominant control and subordinate incentives, as advanced by transactional skew theory. Skew was not lower on parasitised nests, as would be expected if parasites were forced to cede more reproduction in return for subordinate cooperation. Moreover, the fact that many subordinates without offspring nonetheless invested significantly in reproduction provides evidence of a continuing struggle over reproduction, which is not consistent with either concessions models of skew or the allocation of reproduction based on conventions. In contrast, tug-of-war models receive some support, though this is not clear-cut. In particular, while the higher skew on parasitised nests supports a tug-of-war over reproduction, an individual's reproductive share was not related to its RHP. The finding that subordinate reproductive investment was negatively correlated with RHP is also consistent with a tug-of-war. However, ovarian development was not lower among subordinates in parasitised colonies, which would be expected if RHP asymmetries determine a subordinate's investment in reproduction. Overall, a tug-of-war over reproduction remains the most likely scenario, based on the higher skew on parasitised groups, the negative correlation between subordinate reproductive investment and dominant RHP, and the observations of egg-laying and egg replacement among group members.

7.5 Summary

In this chapter, I tested the predictions of competing models of reproductive skew using social parasitism as a means of inducing variation in relatedness and RHP between group members. I found no evidence for reproductive partitioning based on concessions, and only limited support for a tug-of-war over reproduction. Crucially, the strength of these conclusions rests on the ability of individuals to detect and respond to variation in

relatedness following replacement of the host dominant by the parasite. This highlights the importance of information gathering to reproductive skew theory, and more generally, to mechanisms of conflict resolution within animal societies.

Chapter 8: Concluding remarks

In this chapter, I briefly summarise the main findings presented in this thesis. I then relate these findings to more general ideas about information gathering in conflict resolution and suggest potential avenues for future research.

8.1 Status signalling in *P. dominulus*

8.1.1 Summary of findings

In North America, there is evidence that *P. dominulus* clypeal patterns function as status signals (e.g. Tibbetts & Lindsay 2008; Tibbetts *et al*. 2010). However, previous studies in Europe have largely failed to support this idea (Cervo *et al*. 2008; Zanette & Field 2009). In this thesis, I have presented evidence that clypeal patterns do not function as signals of status in a Spanish population (Chapter 3) and that the brokenness of the pattern does not correlate with survival, hierarchical rank or reproductive success in this population (Chapter 4). I have also provided some evidence suggesting that the differences in clypeal pattern variability between populations may be a result of abiotic factors, specifically temperature, affecting pattern development (Chapter 5). Below, I suggest further areas of research that may help to resolve the seemingly contradictory findings of this study and previous work by Tibbetts and co-workers.

8.1.2 Suggestions for future research

The importance of clypeal patterning in wild populations

Brokenness was not related to quality in the Spanish population, as estimated by survival, dominance or reproductive success. This study is the first to examine the relationship between brokenness and quality in a wild population. As I argue in Chapter 4, data on the relationship between clypeal patterning and fitness from other populations are needed. In the North American populations, Tibbetts *et al.* have shown that brokenness sometimes predicts the outcome of agonistic interactions in the lab (Tibbetts & Dale 2004; Tibbetts *et al*. 2011a). However, such interactions bear little resemblance

to those observed in the wild (see Chapter 1). In the absence of a clear demonstration that individuals with more broken patterns enjoy a fitness advantage in wild American populations, the adaptive significance of clypeal patterning remains uncertain.

Exploring the development of the clypeal pattern

Research on status signalling in *P. dominulus* would also benefit from a better understanding of the processes affecting the development of the clypeal pattern. In this thesis, I have provided evidence that temperature can affect the amount of melanin laid down on the clypeus, and other studies have shown that larval nutrition has an impact upon pattern development (Tibbetts & Curtis 2007; Tibbetts 2010). However, the physiological processes governing pattern development are still unknown (see Chapter 5). Identifying these processes is likely to provide insights into the type of information that is conveyed by the clypeal pattern. For example, Tibbetts & Banan (2010) have suggested that brokenness may be related to levels of juvenile hormone (JH). In other insects, JH is known to be important in determining melanisation (Nijhout 1999) and may also regulate expression of the clypeal pattern in *P. dominulus*. Moreover, studies of the vertebrate melanocortin system have indicated that melanin-based coloration and aggression are likely to be under pleiotropic control via a shared hormonal pathway (reviewed in Ducrest *et al*. 2008). In *P. dominulus*, JH may provide a similar link between agonistic ability and clypeal patterning, which could be important in stabilising the brokenness signal and preventing cheating.

An understanding of the processes underpinning pattern development may also help to clarify the aspect of the pattern that conveys quality information (i.e. the active signalling component). While Tibbetts & Dale (2004) argue that the active signalling component of the pattern is its 'brokenness' (see Chapter 1), there is considerable ambiguity about what is measured by the brokenness index. Tibbetts & Dale (2004) suggest that brokenness captures the amount of disruption in the pattern. However, as a measure of disruption it is neither simple nor intuitive compared with, for example, the number of black spots on the clypeus, or else the perimeter of the black spots bordering the yellow background. In fact, the brokenness index is a highly derived metric that does not describe any specific element in the pattern. Rather, it is determined by a

number of different elements of the pattern: all else being equal, brokenness will increase with increasing number of clypeal spots, decreasing size of spots, increasing disruption (i.e. amount of yellow pigment) within spots and increasing asymmetry of spots. Justifying their preference for the brokenness index over simpler measures of pattern disruption, Tibbetts & Dale (2004) state that brokenness 'is a particularly useful parameter because it collapses a wasp badge's degree of advertised dominance into a singular variable'. However, while brokenness may indeed be a singular variable, I would argue that, for the reasons given above, it is neither a particularly intuitive nor meaningful one. Moreover, the sensitivity of the brokenness index to many different elements within the clypeal pattern means that brokenness is not a very useful measure for understanding the processes regulating the expression of the clypeal pattern. However, by studying the formation of the pattern in greater detail, it should be possible to identify more basic, tangible elements of the pattern whose development is affected by factors such as JH or nutrition. Knowledge of how these elements respond to variation in environmental and genetic factors should provide greater insights into the relationship between individual quality and clypeal patterning, as well as pointing to the precise features of the pattern involved in signalling this quality to others.

An understanding of how specific elements of the pattern are influenced by genetic and environmental factors may also yield insights into the evolutionary processes that shape the pattern, and in particular the process(es) responsible for generating variation in the signal value of the pattern between populations. As I suggest in Chapter 5, variation in signal value may be linked to differences in the variability of clypeal patterns between populations. Abiotic factors such as temperature may act to constrain pattern variability (see Chapter 5); however, other processes could also be important. For example, Tibbetts (2010) has shown that both the size and brokenness of the pattern have a significant heritable component in the American populations. Drift, local adaptation or genotype x environment interactions may therefore also play a role in maintaining variation in the pattern between populations (Mitchell-Olds *et al*. 2007). Examining the genetic basis of pattern expression may provide an insight into these processes, as well as indicating the direction of possible changes in signal value, which currently remains unclear *i.e.* was the signal lost in the ancestral populations and retained in the invasive populations, or is status signalling an evolutionary novelty in American populations?

Tests of status signalling in other species

Despite extensive research in *P. dominulus*, there has been little exploration of status signalling in other *Polistes* species. Indeed, efforts to explore status signalling between conspecifics in other species is limited to a single study on the tropical paper wasp *P. satan*. Tannure-Nascimento *et al*. (2008) have suggested that the relative proportion of brown vs. black cuticle on the head of *P. satan* signals fertility and/or status. Unfortunately, the data presented by Tannure-Nascimento *et al*. (2008) do not permit a rigorous test of this hypothesis, as they do not appear to exclude the possibility that such markings function in individual recognition.

Studies of status signalling in other *Polistes* species will be important for determining how widespread this behaviour is in the genus, but will also be useful for exploring the cognitive processes underpinning the use of visual signals in these wasps. For example, Gronenberg *et al*. (2008) have compared the anatomy of the *P. dominulus* brain with that of several other *Polistes* species. The authors found no difference between species in the size of the mushroom bodies or optic lobes (both areas associated with visual processing in insects), leading Gronenberg *et al*. to suggest the that neural machinery necessary for visual processing of clypeal patterns may not be very elaborate and may in fact be co-opted from mechanisms that have evolved for other visual tasks (Gronenberg *et al*. 2008). However, this conclusion rests on the assumption that two of the other species examined in the study (*P. arizonensis* and *P. flavus*) do not use visual signals, and there is currently no evidence to support this idea. Determining whether these and other species use status signals will hopefully allow for a more powerful test of the relationship between signalling and specific features of neural anatomy, and may also be useful for determining how information is extracted from clypeal patterns and subsequently processed by receivers. In *P. dominulus*, Tibbetts & Dale (2004) have argued that brokenness is an effective channel through which to signal information. However, in other species with variable clypeal patterns (e.g. *P. exclamans* and *P. gallicus*), it is the size of the clypeal spot that varies between individuals, not the amount of disruption (J. P. Green, pers. obs.). Determining the signal value of these patterns may thus provide insights into the mechanisms wasps use to discriminate particular features of patterns, which has to date been little explored (Avargues-Weber

et al. 2011). Such studies would also provide a better understanding of the cognitive processes associated with status signalling. For example, do individuals display innate responses to particular kinds of patterns (e.g. highly disrupted patterns) as suggested by Gronenberg *et al*. (2008), or do they instead learn specific associations between a rival's agonistic ability and its clypeal patterning, which could then be generalised to other encounters with unfamiliar individuals (Avargues-Weber *et al*. 2011)?

8.2 Information gathering in host-parasite interactions

8.2.1 Summary of findings

Much recent attention has been given to the abilities of animals to gather information about their own and their rival's abilities during contests (Arnott & Elwood 2009). However, nothing is known about the scope for information gathering in contests between heterospecific rivals. In this thesis, I explored the potential for rival assessment in usurpation contests between *P. dominulus* and a social parasite, *P. semenowi* (Chapter 6). My results of indicate that rivals do not make use of information about rival ability when deciding whether to fight or withdraw. Rather than this pointing to limitations in the information-gathering abilities of hosts and parasites, I suggest that the high value placed by both contestants on the resource, together with the potential for large asymmetries in RHP fuelling a desperado strategy among hosts, may result in selection favouring escalated fighting over conflict resolution strategies.

8.2.2 Suggestions for future research

Information gathering between heterospecifics

In this thesis, I explored rival assessment between two *Polistes* species. Within the social Hymenoptera, other socially parasitic species engage in aggressive interactions with hosts – for example, social parasites of the genus *Vespula* battle with the host queen in a bid to replace her as the reproductive within the colony (e.g. Reed & Akre 1983), while many slave-making ant species engage hosts in aggressive fighting while

attempting to gain access to their brood (D'Ettorre & Heinze 2001). However, in these contests the stakes are likely to be very high, with hosts suffering a dramatic loss of fitness following successful invasion by the parasite. Therefore, the lack of rival assessment that I observed in aggressive interactions between *P. dominulus* and *P. semenowi* may be a general feature of usurpation conflicts between hosts and social parasites in the Hymenoptera, with the high value of the nest favouring escalation over information gathering and rival assessment.

However, there are other contexts in which selection may favour information gathering between heterospecifics. One such context is competition between species for access to food sources (e.g. Watts *et al*. 2010; Lichtenberg *et al*. 2011). For example, Lichtenberg *et al*. (2011) have suggested that stingless bees (*Trigona* spp.) can detect the recruitment pheromones laid by heterospecifics, and use this information to avoid food sources visited by individuals of more dominant species. Eavesdropping on heterospecifics may also minimise the cost of competition for food between lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) (Webster *et al*. 2010; Watts *et al*. 2011). Results of play-back experiments indicate that both species can detect the presence of heterospecific competitors by eavesdropping on vocalisations (Webster *et al*. 2010). Moreover, there is evidence that behavioural responses to lion vocalisations vary among hyenas (Watts *et al*. 2010). This may point to a form of mutual assessment, whereby a hyena uses information about its own ability and the ability of a heterospecific rival (e.g. a lion) to assess the costs and benefits of approaching versus withdrawing (see Arnott & Elwood 2009). However, detailed studies of contest behaviour, including the use of vocalisations and other behaviours at different stages of the contest, will be necessary to explore the possible assessment rules underlying contests between these species.

Importantly, information gathering between heterospecifics is not limited to competitive contexts. There is growing evidence that heterospecifics constitute an important source of information for animals when selecting suitable breeding or foraging sites (reviewed in Valone 2007). For instance, three-spined sticklebacks (*Gasterosteus aculeatus*) and nine-spined sticklebacks (*Pungitius pungitius*) have been shown to use information about the foraging success of heterospecifics when selecting foraging patches (Coolen *et al*. 2003). Individuals are also known to gather information about the risk of

predation by eavesdropping on the alarm calls of other species (e.g. Magrath & Bennett 2011). Although the use of such 'public' information has traditionally focused on information transfer between conspecifics, heterospecifics that occupy similar niches and depend upon similar resources provide a potentially valuable source of information, which can then be incorporated within an individual's decision-making process (Valone 2007; Magrath & Bennett 2011).

8.3 Information gathering and reproductive partitioning in animal societies

8.3.1 Summary of findings

Within social groups, there is often intense competition over reproduction, the outcome of which may determine the scope for cooperation between group members, as well as the stability and productivity of the group. Transactional models of reproductive skew assume that individuals negotiate reproductive shares based on the costs and benefits of group living, whereas tug-of-war models assume that reproductive partitioning is achieved through direct competition between group members. Intra-group relatedness is thought to be an important determinant of skew in transactional models, but does not greatly affect skew in tug-of-war models. Previous studies of skew in *P. dominulus* have found no effect of relatedness on skew, arguing against the existence of reproductive transactions in this species (Queller *et al*. 2000; Liebert & Starks 2006). However, it is unclear whether *P. dominulus* is able to detect relatedness, or whether they use other, more general cues as a guide to relatedness (such as nest-mate cues). If individuals are unable to acquire precise information about relatedness, previous tests of reproductive skew models that used the relationship between relatedness and skew as a means of testing between competing models may have been flawed. In this thesis, I attempted to avoid this problem by using social parasitism as means of generating detectable variation in relatedness within groups (Chapter 7). Skew on both parasitised and unparasitised nests was high, though slightly higher on parasitised nests. This result indicates that reproductive partitioning within *P. dominulus* colonies is not determined via reproductive transactions, but rather points to a tug-of-war over reproduction between group members.

8.3.2 Further remarks on information gathering and reproductive skew

The ability of group members to detect and respond to features of the environment (both social and non-social) is critical to transactional models of reproductive skew for two reasons. First, information is needed to assess the costs and benefits of group living versus independent breeding (e.g. Nonacs 2006; Field & Cant 2009). For example, individuals need to obtain information about the level of ecological constraints on independent breeding in order to assess the advantages of group living. Evidence that individuals can detect and respond to changes in ecological constraints comes from a study of the cooperatively breeding cichlid *Neolamprologus pulcher* by Bergmüller *et al*. (2005), who found that helpers deserted groups in response to an increase in nest site availability. In the case of primitively eusocial wasps, Field & Cant (2009) have argued that the ability to move freely in the environment may allow individuals to acquire information about ecological constraints. However, as Field & Cant (2009) point out, dominant individuals rarely leave the nest and may therefore have little opportunity to gather information about a subordinate's scope for independent nesting. The size of reproductive concessions offered by the dominant may therefore be insensitive to variation in ecological constraints, which is consistent with recent findings that skew does not vary with the magnitude of constraints (Langer *et al*. 2004; Heg *et al*. 2006).

Accurate assessment of the costs and benefits of group living also requires that individuals gather information about other group members. For example, transactional models predict that individuals will vary their demand for reproduction in response to their perceived relatedness to the dominant. This may be determined directly, or else through the use of general cues, as appears to be the case in *P. dominulus*. The asymmetry in RHP between a dominant and its subordinate is also important in transactions over reproduction, as it determines the size of the peace incentive required to dissuade the subordinate from escalated conflict with the dominant (Reeve & Ratnieks 1993). Therefore, negotiations over reproduction may favour the evolution of strategies for assessing the RHP of negotiating partners, for example via signals or cues. Indeed, given that the threat of escalation depends on asymmetries in dominant-subordinate RHP, such interactions may select for mutual assessment, with individuals gathering information about their own abilities as well as those of their partner (Arnott

& Elwood 2009). At present, however, little is known about the role of RHP assessment in reproductive partitioning, and this may be an interesting area for future research.

The second reason that information gathering is important in transactional skew models is that the stability of transactions depends on individuals detecting and responding to attempts to cheat by the party controlling reproduction (Clutton-Brock 1998; Kokko 2003; Field & Cant 2009). In particular, where cooperation is obtained with the promise of incentives (or the threat of eviction stayed by the promise of restraint), an individual must be able to detect efforts by the other party to renege on the deal, or else the association will break up (Kokko 2003). One obvious way for a dominant to cheat a subordinate of its concession is to destroy the subordinate's offspring (Clutton-Brock 1998). Offspring destruction in *Polistes* occurs commonly via differential oophagy, and there is evidence that in *P. dominulus* dominants remove eggs laid by subordinates (e.g. Liebig *et al*. 2005). Overall, the potential for imperfect information or imperfect information-gathering abilities to limit the extent of cooperation in social species represents a fruitful topic for future research. For example, what is the effect of impairing an individual's ability to acquire information about relatedness or ecological constraints, or even the occurrence of cheating, on its propensity to cooperate and the share of reproduction that it receives if it remains within the group?

8.3 Final remarks: limitations on information gathering

In order to make optimal (i.e. fitness-enhancing) decisions, animals must acquire information about their environment (Danchin *et al*. 2008). However, a recurring theme of this thesis is that there is a limit both to the information that is available to animals and to the usefulness of information in particular situations. This can be seen in the studies of status signalling in *P. dominulus*, where the use of status signals appears restricted to certain populations, possibly as a result of constraints on signal evolution imposed by environmental conditions. The limits to information gathering are also made clear in the case of kin discrimination in *P. dominulus*, which I discuss in Chapter 7. Co-foundress groups contain significant numbers of unrelated females. Although these unrelated females achieve greater fitness through joining groups than by nesting

alone, they would gain substantially higher benefits by directing their help towards relatives (Leadbeater *et al*. 2011). Why then do females not seek out sisters with whom to nest? Part of the answer may lie in the fact that *P. dominulus* does not seem able to detect variation in relatedness between individuals. Females do, however, discriminate between nest-mates and non-nest-mates; providing that nest-mate groups contain related individuals, this should allow individuals to target helping behaviours towards relatives. One potential reason why unrelated females end up nesting together is that gynes from a number of different nests may seek shelter in the same hibernaculum over winter, resulting in mixing of nest-mate recognition cues between different sister groups (Dapporto *et al*. 2004). In this case, therefore, the ability to gather accurate information about kinship carries with it significant fitness benefits, yet in practice may be impossible to achieve, leading to sub-optimal joining decisions among co-foundresses.

Information may thus be difficult to acquire in some situations; in others, however, information may be readily available but of little value. For example, models of contest behaviour predict that escalation should be favoured over rival assessment in competition over valuable resources (e.g. Enquist & Leimar 1987, 1990). In contests between host wasps and invading social parasites, rival assessment may offer few benefits in the face of severe fitness costs associated with relinquishing the resource. Escalation and fatal fighting have also been observed in competition for access to females between male fig wasps (*Sycoscapter* sp.), which proceeds without any rival assessment (Moore *et al*. 2008). The findings of both studies indicate that rival assessment, even if possible, may not always be profitable. In both cases, however, individuals are assumed to gather information about the value of the resource, which then establishes their level of motivation and aggression during the contest (Arnott & Elwood 2008). Thus, in the event that assessment is discarded in favour of dangerous combat, information gathering remains a powerful force shaping conflicts between animals.

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Appendix A. Summary of research to date into status signalling in *P. dominulus*.

Study	Main findings	Conclusions/Comments
North America		
Tests of signal function		
Tibbetts & Dale (2004)	Brokenness predicts body size and outcome of dyadic contests. Pattern manipulations elicit aggressive responses from receivers	Evidence of social costs (but no direct evidence for status signalling via pattern brokenness - see main text)
Tibbetts & Lindsay (2008)	Wasps more likely to forage near low-spot guard than high-spot guard.	Rival assessment based on clypeal pattern during competition over food
Tibbetts (2008)	Hunger level predicts willingness to approach high-spot guard but not low-spot guard.	Receiver responses to brokenness signal depend on context (here, resource value).
Tibbetts *et al.* (2010)	Wasps more likely to forage near low-spot guards when own brokenness score is low.	Individuals incorporate information about own quality during rival assessment.
Tibbetts & Izzo (2010)	Pattern manipulations elicit aggression from receivers, but not when coupled with behavioural manipulations.	Further evidence of social costs, and support for the incongruence hypothesis
Quality basis of clypeal patterns		
Tibbetts (2006)	Pattern brokenness positively correlated with date of emergence.	Brokenness reflects offspring quality, which increases over the season.
Tibbetts & Curtis (2007)	Colonies that received additional food produced offspring with higher pattern brokenness.	Brokenness reflects quality (but offspring with more broken patterns found to be smaller).
Tibbetts (2010)	Colonies that received additional food produced offspring with higher pattern brokenness.	Brokenness reflects quality, and is principally determined by rearing environment.
Tibbetts & Banan (2010)	Brokenness predicts survival following JH application.	Evidence for differential survival costs associated with signal elaboration.
Tibbetts & Izzo (2010)	Brokenness predicts investment in egg production following JH application.	Reproductive investment is made based on assessment of own quality.
Tibbetts *et al.* (2011a)	Brokenness predicts outcome of dyadic contests (but only in 1 out of 2 years). Brokenness is positively correlated with JH titre.	JH mediates link between brokenness and RHP.
Tibbetts *et al.* (2011b)	Brokenness predicts timing of post-diapause activity.	Individuals with more broken patterns emerge from diapause earlier and are more likely to become dominant.

Appendix A Continued.

Study	Main findings	Conclusions/Comments
Tibbetts (2007)	Clypeal patterns do not predict tenure of early workers on the nest.	Individual quality does not inform cooperative decisions.
Tibbetts & Shorter (2009)	Clypeal patterning predicts outcome of nest usurpation contests.	Clypeal patterns reflect RHP (but small sample size and pattern manipulations not performed).
Italy		
Cervo *et al*. (2008)	Brokenness does not predict rank in co-foundress groups. Brokenness does not predict survival during diapause. Brokenness does not predict parasitism by Strepsiptera endoparasites.	Brokenness does not predict quality within *P. dominulus*' native range.
Spain		
Zanette & Field (2009)	Size of the clypeal spots is a weak predictor of rank in co-foundress groups, but has no effect when analysed together with other potential determinants of rank.	Large clypeal spots are neither sufficient nor necessary for the attainment of high social rank (but study based on small number of wasps with clypeal spots).
Ukraine		
Rusina *et al*. (2006)	Usurpers of single-foundress nests have more broken patterns than residents, and brokenness tends to predict outcome of usurpation attempts.	Pattern brokenness may signal RHP.
Rusina *et al*. (2007).	Brokenness is positively correlated with body size among co-foundresses. Brokenness is not correlated with rank in co-foundress groups.	None

Appendix B. Characterisation of the nine microsatellite loci in *Polistes dominulus* on which maternity assignment in Chapters 4 and 7 was based. [1]Number of unrelated *P. dominulus* individuals genotyped for primer development, belonging to a single population collected at Conil de la Frontera, Cádiz, Spain in 2009. Table adapted from Leadbeater *et al*. 2010 (online supporting information: S1).

Locus	EMBL accession number	Reference	Repeat motif in source species	Forward and reverse primer sequences (5'-3')	N[1]	Expected allele size (bp)	Observed allele sizes (bp)	No. of alleles	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)
Pbe128TAG	U64637	A	$(TAG)_{12}$	F: CCGATATCCGTGCCAGTGATAC R: GCTACCGCGACTGCTGTCC	26	170	128-197	15	0.81	0.83
Pdom1jc	AF155596	B	$(CAG)_9TAG(CAG)_5$ $(CAT)_5GGCAC(CAG)_3$	F: TCGGCTGATTTGTCAATACG R: ATTTAATCGCGAACGGTGTC	26	275	243-290	19	0.69	0.76
Pdom2jc	AF155597	B	$(AAG)_8CG(AAG)_2$	F: AGACCCACCAGCTCCTCTC R: TCTTCGTTCCTTAAGCTTACAATG	26	180	165-191	11	0.58	0.69
Pdom25jc	AF155600	B	$(AAG)_{11}$	F: CCGCGTTAACGATGAATG R: TGGAAACGTAAGTCCACTCG	26	142	120-147	9	0.69	0.65
Pdom7	AF155598	C	$(CAG)_9TAG(CAG)_5$ $(CAT)_5GGCAC(CAG)_3$	F: CACTGTATTGTCCTACGGTGGTCC R:GCGAGAACCTGTACTCAAAACAAAC	23	160	154-179	9	0.65	0.67
Pdom20	AF155599	C	$(CAT)_{18}$	F: TTCTCTGGCGAGCTGCACTC R: AGATGGCATCGTTTGAAAGAGC	27	236	210-372	29	0.89	0.91
Pdom127b	AF155610	C	$(AAT)_{13}...(AAT)_6$ $AA(AAT)_4AAC(AAT)$	F: TCCCCCGTTTTTGGTCCTTG R: GGGAGAGAATCGTGCCTTTTC	24	119	106-171	18	0.92	0.90
Pdom140	AF155613	C	$(TAG)_9$	F: GCTTTTCCCTTATTTTCCCG R: CGTGTTCGTATATTCCTGTAACG	27	192	196-244	21	0.85	0.84
Pdom122	Af155609	D	$(AAT)_{10}GAA(AAT)_4GAA(AAT)_9$	F: CGAACGATAAGTGGAAATAACG R: CTCTCTTCGCGAGACCATC	25	234	188-286	35	0.96	0.98

References for microsatellite sequences and primer sets: A. Strassmann *et al*. 1997; B. Leadbeater *et al*. 2010; C. Henshaw 2000; D. Leadbeater *et al*. 2011.

Appendix C. Characterisation of the eight microsatellite loci in *Polistes semenowi* on which maternity assignment in Chapter 7 was based. [1]Number of *P. semenowi* individuals genotyped, collected from sites around Conil de la Frontera, La Zarzuela and Zahara de los Atunes, Cádiz, Spain in 2010.

Locus	EMBL accession number	N[1]	No. of alleles	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Hardy-Weinberg Equilibrium p value	Estimated null allele frequency
Pbe128TAG	U64637	30	6	0.33	0.31	1	-0.08
Pdom1jc	AF155596	30	9	0.67	0.60	0.75	-0.08
Pdom2jc	AF155597	27	4	0.13	0.18	1	-0.04
Pdom25jc	AF155600	30	3	0.10	0.10	1	-0.02
Pdom7	AF155598	26	9	0.85	0.81	0.69	-0.04
Pdom20	AF155599	30	13	0.80	0.87	0.19	0.03
Pdom140	AF155613	20	10	0.75	0.93	0.007	0.03
Pdom122	Af155609	25	17	0.96	0.98	0.71	-0.03

Appendix D. Publications arising from, and relating to, work presented in this thesis

Green, J.P. & Field, J. 2011. Assessment between species: information gathering in usurpation contests between a paper wasp and its social parasite. *Animal Behaviour*, **81**, 1263-1269.

Green, J.P. & Field, J. 2011. Inter-population variation in status signalling in the paper wasp *Polistes dominulus*. *Animal Behaviour*, **81**, 205-209.

Leadbeater, E., Carruthers, J. M., Green, J. P., van Heusden, J. & Field, J. Unrelated helpers in a primitively eusocial wasp: is helping tailored towards direct fitness? *Plos One*, **5**, e11997.

Leadbeater, E., Carruthers, J. M., Green, J. P., Rosser, N. & Field, J. 2011. Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science*, **333**, 874-876.

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Assessment between species: information gathering in usurpation contests between a paper wasp and its social parasite

Jonathan P. Green*, Jeremy Field

School of Life Sciences, University of Sussex

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Costs associated with escalated fighting may be minimized where individuals are able to gather information regarding the likely outcome of conflicts. In particular, the ability to assess resource-holding potential (RHP) has been shown to be important in determining the dynamics of animal contests. While assessment rules have been investigated in contests in a range of species, little is known about the potential for assessment in contests between species. We examined the role of assessment in usurpation contests between the paper wasp *Polistes dominulus* and the social parasite *Polistes semenowi*. First, we investigated whether parasite clypeal patterns function as signals of RHP by staging contests with parasites in which the clypeal pattern was concealed with paint. Second, we examined the importance of body size as a determinant of RHP. Finally, we explored whether individuals use information about their own RHP, and that of their rivals, in deciding when to withdraw. We found no evidence that parasite clypeal patterns act to signal RHP to hosts: initial fights were neither longer nor more intense when the patterns were concealed. We also found no evidence for RHP assessment during contests: although body size predicted contest outcome, fight duration and intensity were not significantly related to either winner or loser size. We suggest that the high value of the nest to both parties, combined with the potential for 'divisive' asymmetries in RHP between hosts and parasites, may result in selection for escalated conflict over rival assessment during usurpation fights.

Contests over resources are widespread in nature, both between and within species. The outcomes of contests can have important fitness consequences, not only in terms of the resources gained or lost, but also in terms of the energetic cost of fighting and the risk of injury (e.g. Clutton-Brock et al. 1979; Mann et al. 2001; Briffa & Sneddon 2007). To minimize these costs, individuals may make strategic decisions based on their own resource-holding potential (RHP) and that of their rival (Arnott & Elwood 2009). Information acquired during contests about an individual's own performance, as well as its opponent's performance, is valuable as it allows rivals to assess the costs and benefits of continued fighting versus withdrawing from the contest (Arnott & Elwood 2009). Alternatively, or additionally, animals may assess rival ability prior to contests by attending to cues or signals of RHP (Maynard Smith & Harper 2003).

The type of information gathered by animals during contests, as well as the conditions under which information gathering occurs, has been the subject of much theoretical and empirical work (reviewed in Arnott & Elwood 2009). For example, by examining the relationship between winner and loser RHP and contest dynamics, it has been possible to deduce the likely assessment strategies used during contests (Taylor & Elwood 2003; Arnott & Elwood 2009). Information gathering may be limited to an assessment of an individual's own performance during a fight, with individuals withdrawing when an upper threshold is reached (self-assessment). Alternatively, individuals may base the decision to retreat on information about their own and their rival's RHP (mutual assessment). Furthermore, information gathered about other aspects of the contest, in particular the value of the contested resource, has been shown to have a critical role in shaping fight dynamics (Arnott & Elwood 2008). Resource value can affect an individual's fighting strategy via its effect on motivation: in cases where resource assessment is possible, individuals that place a greater value on the resource are expected to fight for longer and/or at greater intensities to secure the resource than individuals with lower motivation (Arnott & Elwood 2008). Indeed, in situations where the perceived value of the resource is so great that the benefits of winning outweigh the cost of escalated fighting, individuals are expected to persevere, irrespective of asymmetries in RHP (Enquist & Leimar 1990). While there is evidence that high resource value favours escalated fighting over self- and rival assessment in certain situations (e.g. Moore et al. 2008), in general some form of assessment based on signals/cues or performance during contests is expected to reduce the costs of fighting, and has

* Correspondence: J. P. Green, School of Life Sciences, John Maynard Smith Building, University of Sussex, Falmer, Brighton BN1 9QG, U.K.
E-mail address: j.p.green@sussex.ac.uk (J. P. Green).

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been demonstrated in a wide range of taxa (e.g. Stuart-Fox 2006; Prenter et al. 2008; Tibbetts et al. 2010).

To date, studies of animal contests have focused largely on fights between conspecifics for resources. Members of the same species tend to occupy very similar ecological niches and therefore experience intense competition with one another over the limited resources available. Analysis of conspecific contests is aided by the fact that resources valuable to both contestants are readily identified, and factors determining RHP will be common to both contestants. Perhaps for these reasons, contests over resources between members of different species have been largely neglected (but see Macdonald et al. 2007; Tanner & Adler 2009). However, such contests play an important part in determining an individual's access to resources, with competition occurring between species, for example, for food and breeding sites (e.g. Becerril-Morales & Macías-Ordóñez 2009; Strubbe & Matthysen 2009). Thus, it is important to know which factors influence the outcome of contests between heterospecifics, and to what extent individuals are able to gauge the competitive abilities of heterospecific rivals.

Social parasites of primitively eusocial and eusocial Hymenoptera offer a good opportunity to study assessment strategies used in contests between heterospecifics. Following invasion of the host nest, social parasites take up residence with the hosts, on whom they depend to rear their offspring. Social parasites deploy a variety of deceptive strategies to subvert the host recognition system and so gain entry to the host nest, including chemical mimicry and dilution of cuticular compounds (chemical insignificance; Lorenzi 2006). However, a number of social parasites in the Vespinae and Polistinae adopt an aggressive usurpation strategy, engaging in prolonged fights with hosts (Reed & Akre 1983; Cervo 2006). While these aggressive interactions are well described for a number of species (e.g. Reed & Akre 1983; Zacchi et al. 1996), there has been little research into the factors determining fight outcome and dynamics, as well as the possible mechanisms of rival assessment used by hosts and parasites during usurpation.

In this study, we focused on the aggressive interactions that characterize host usurpation attempts by *Polistes semenowi*, one of three obligate social parasites within the paper wasp genus *Polistes*. *Polistes semenowi* is considered a specialist parasite of the European paper wasp, *Polistes dominulus* (Cervo 2006). *Polistes semenowi* targets host nests in the late pre-emergence phase of the colony cycle, when colony foundresses are the only adults present. The parasite invades aggressively, fighting with host foundresses until they submit (Zacchi et al. 1996). The original dominant foundress often remains in the colony, although her role as the principal egg-layer is now assumed by the parasite. In common with other *Polistes* social parasites, *P. semenowi* presents several morphological specializations, including enlarged femora and thickened mandibles, which are thought to enhance fighting ability (Cervo 2006).

In addition to the above adaptations for fighting, *P. semenowi* has conspicuous black patterning on the clypeus. Recent research into the function of clypeal patterns in the host species, *P. dominulus*, has demonstrated that these patterns play an important role in rival assessment (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008). *Polistes dominulus* clypeal patterns are argued to function as status signals (or 'badges') that signal RHP (Tibbetts & Dale 2004), and there is evidence that they are useful in settling contests between unfamiliar rivals by making asymmetries in RHP apparent when rivals meet (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; but see Green & Field 2011). Although somewhat larger and less variable than host patterns, the clypeal patterns of the parasite may similarly function as signals of status. In the host, the active signal component of the pattern is its disruption, or 'brokenness' (Tibbetts 2010). In the parasite, however, melanin deposition is uniform across the clypeus and extends down to the mandibles. Noting this pattern, Ortolani et al. (2010) have alternatively proposed that the parasite clypeal patterns act as amplifiers (Taylor et al. 2000), highlighting a signal of RHP based on mandibular width. In an experiment in which heads of a second *Polistes* parasite, *Polistes sulcifer*, were presented to *P. dominulus* hosts, hosts showed more aggression towards heads painted to conceal the clypeal pattern (Ortolani et al. 2010). This finding raises the possibility that the clypeal patterns of *P. semenowi* function in parasite assessment by hosts during usurpation contests. If parasite clypeal patterns function as signals of RHP, either as status badges or amplifiers, then they may assist usurpation by reducing host aggressive responses.

In this study, we explored the potential for rival assessment in usurpation contests between *P. semenowi* and *P. dominulus* hosts. First, we tested the hypothesis that parasite clypeal patterns function to signal RHP in usurpation contests with hosts. We tested this by manipulating parasite clypeal patterns and observing the effect of the manipulations on the duration and intensity of fights with hosts. Signals of RHP based on clypeal patterns provide a potential mechanism for rival assessment prior to fighting; alternatively (or additionally), individuals may use information about rival RHP acquired during contests when deciding whether to persevere or retreat. Body size in *Polistes* wasps is often associated with social rank (Turillazzi & Pardi 1977; Cervo et al. 2008) and in *P. dominulus* body size affects the outcome of both intraspecific (Tibbetts & Shorter 2009) and interspecific (Ortolani & Cervo 2010) usurpation attempts. We therefore also analysed contest dynamics and outcome with respect to rival size to establish first whether size is a determinant of RHP in *P. dominulus*–*P. semenowi* contests, and second whether size information is used in strategic assessment during fights, as has been reported in other taxa (e.g. Morrell et al. 2005; Prenter et al. 2008).

METHODS

Polistes dominulus colonies parasitized by *P. semenowi* were located at sites around Conil de la Frontera and Zahara de los Atunes, Cádiz Province, Spain, in early May 2010. Because of the relative rarity of parasites in our study population (typically only 2% of host nests are parasitized), several hundred colonies had to be inspected to obtain the sample of 31 parasites used in the experiment. Parasites were removed from their host nests and placed in individual containers for 24 h prior to usurpation trials. To control for effects of resource value on the motivation of parasites to attack or hosts to defend, we presented parasites with similarly sized host colonies, in terms of nest size (mean $\pm$ SE $= 73.16 \pm 3.33$ cells), number of adult hosts (2.87 ± 0.10) and number of pupae (12.65 ± 1.17). Using intensive field surveys, we identified target host colonies with no prior history of parasitism; however, it is possible that colonies experienced brief usurpation attempts that were missed by the surveys. Therefore, to ensure that hosts and parasites had not previously interacted, parasites were presented with a host colony collected at least 3 km from where the parasite was found.

Parasite Manipulation

Prior to usurpation trials, parasites were marked with a spot of paint on the thorax to aid identification during video analysis. To test the importance of the clypeal pattern for agonistic interactions with hosts, parasites were randomly assigned to one of two treatment groups (Fig. 1). In Treatment 1 ($N = 14$), yellow paint was added to cover the black clypeus mark completely. In Treatment 2 ($N = 17$), yellow paint was added to the yellow genae to control for the presence of paint, the odour and spectral reflectance of which

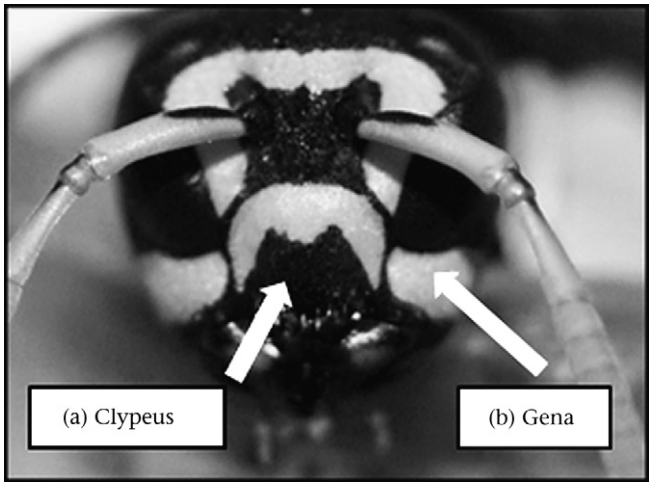

Figure 1. Head of *P. semenowi* (♀), with arrows indicating the (a) clypeus and (b) genae painted yellow in the experimental and control treatments, respectively.

are expected to differ from those of the cuticle. While it is possible that the presence of paint in general alters the behavioural response of the receiver, our experimental design allowed us to test for a specific effect of the parasite clypeal pattern on host behaviour over and above any general response to the presence of paint. Previous observations of manipulated parasites from established nests in the field have shown that such manipulations affect neither the parasite's ability to relocate its nest nor its subsequent behaviour towards hosts (J. P. Green, unpublished data).

Usurpation Trials

Thirty-one usurpation trials were carried out outdoors on warm, bright days between 1300 and 1600 hours (identified by Ortolani & Cervo (2009) as the time of peak activity in a related social parasite, *P. sulcifer*). In each trial, a single parasite was placed in a plastic cage (34×18 cm and 27 cm high) containing a target host nest and allowed to approach the nest and interact with hosts. In those trials where the parasite approached the nest within 2 h, we filmed all fights between parasites and hosts for 1 h following the initial approach (henceforth, 'observation period') using digital camcorders. Each parasite and host colony was used only once in the trials.

Morphological Measurements

Upon completion of usurpation trials all individuals were killed by freezing. Following Tibbetts & Dale (2004) and Ortolani & Cervo (2010), we used head width as a measure of body size. For each wasp, the head was removed and placed on a microscope slide. Head width was measured as the width at the widest point using a 16× binocular microscope. At the same time we recorded the presence or absence of clypeal patterns on the hosts. The presence or absence of clypeal patterns can be used as a simple indicator of host quality (Tibbetts & Lindsay 2008), and relates closely to the active signal component, brokenness: hosts without clypeal patterns have a lower brokenness (i.e. 0) than hosts with clypeal patterns.

Behavioural Analyses

For each trial, we recorded the total number of fights within the observation period. We also recorded the duration of each fight, with a pause of more than 30 s signifying the end of a fight. To investigate the effect of parasite clypeal patterns on aggressive interactions, we used data only from the initial encounter between parasite and hosts (i.e. the first fight, following the parasite's initial approach to the nest), as it has been suggested that familiarity between signaller and receiver can confound manipulative tests of signal function (Senar 1999). We obtained three measures from each initial fight: its duration and two measures of its intensity. To assess fight intensity, we distinguished between two classes of agonistic interactions observed during fights, according to likely energetic costs and risk of injury. 'Low-intensity' interactions were darting, antennating, chasing and lungeing (here defined as a rapid movement towards an individual resulting in physical contact). 'High-intensity' interactions were biting, grappling, 'dive bombing' (a behaviour shown by hosts during usurpation attempts in the wild where hosts fly into the parasite, pushing or biting on contact) and wrestling (wasps clasp legs tightly around one another attempting to sting and/or bite opponent). Fight intensity was then estimated in two ways: the number of high-intensity interactions occurring during it, and the total duration of these high-intensity interactions (as a proportion of total fight duration).

Statistical Analyses

All analyses were performed using the software R version 2.9.2 (http://www.r-project.org/). To test the signal function of the parasite clypeal patterns, we compared the duration and intensity of the first fight between the two treatment groups. The effect of the paint treatment on fight duration was analysed using a linear model (LM) with normal errors. Fight duration (log transformed) was fitted as the dependent variable with paint treatment fitted as a categorical independent variable. Recent work has suggested that, in *P. dominulus*, receiver responses to clypeal patterns depend on the RHP of receivers (Tibbetts et al. 2010). As a measure of receiver RHP, we recorded the proportion of hosts with clypeal patterns on each nest (i.e. within each trial). To test whether host responses to the parasite manipulations were dependent upon host RHP, we included the interaction between paint treatment and proportion of hosts on the nest with clypeal patterns as a covariate in the analysis. To examine the effect of parasite clypeal patterns on fight intensity, we compared both the number and duration of high-intensity interactions between treatments. A generalized linear model (GLM) with a quasi-Poisson error distribution was used to analyse the number of high-intensity interactions, as these data were in the form of overdispersed counts. The duration of high-intensity interactions was analysed as a proportion of the total fight duration. As these data also exhibited overdispersion, they were analysed using a GLM with quasibinomial errors. Paint treatment was fitted as an independent variable in both models and, as above, we also included the interaction between paint treatment and the proportion of hosts with clypeal patterns.

To investigate the influence of body size on RHP, we compared the sizes of winners and losers of usurpation contests. Parasites that succeeded in usurping nests were considered winners, and their defeated hosts losers; likewise, parasites that failed to usurp were losers, and their successful hosts winners. Measurements taken from winners and losers cannot be considered as independent because the outcome of a contest (i.e. who wins) is determined by the interaction between the two contestants (Briffa & Elwood 2010). Therefore, to explore the effect of size on contest outcome, we ran a general linear mixed model (GLMM) with normal errors in which 'trial number' was fitted as a random factor. Following Briffa & Elwood (2010), size was fitted as the dependent variable and outcome ('winner' or 'loser') as an independent variable. In the special case of interspecific contests, it is possible that the extent to

which a trait influences contest outcome varies between the two species. To investigate whether the importance of size for contest outcome varies between parasites and hosts, we also fitted the interaction between outcome and contestant species (host or parasite).

Finally, we explored the relationship between winner and loser size and contest duration and intensity to test for possible assessment rules governing usurpation contests. First, we examined the relationship between winner and loser size and the duration and intensity of the first fight. Winners and losers were assigned based on whether the parasite was present on the nest at the end of the fight. The effect of winner/loser size on duration was analysed using a linear model with normal errors. Fight duration (log transformed) was the dependent variable, and winner and loser size the independent variables. As above, fight intensity was analysed as both the number and duration of high-intensity interactions using GLMs with quasi-Poisson and quasibinomial errors, respectively.

Second, we tested for a relationship between winner and loser size and the total number and duration of fights within the 1 h observation period. Here, winners and losers were assigned based on whether the parasite was present on the nest at the end of the observation period. The effect of winner–loser size on total duration was analysed using a linear model with normal errors. Total duration (log transformed) was the dependent variable, and winner and loser size the independent variables. Total number of fights did not follow a normal distribution due to many encounters involving only a single fight. We therefore used a GLM with Poisson errors with number of fights as the dependent variable, and winner and loser size as the independent variables.

For all analyses, model simplification proceeded by backwards deletion of nonsignificant terms until further removals led to a significant ($P < 0.05$) increase in deviance. This was assessed by comparing the models with and without the term in question, using tabulated values of F values for linear models with normal, quasi-Poisson or quasibinomial errors, χ^2 values for linear models with Poisson errors, and log likelihood tests for linear mixed models. Significance levels are reported for the addition of nonsignificant terms, and removal of significant terms, from the minimal adequate model. In all analyses, host head width was calculated as the mean head width of hosts within colonies. Substituting mean host head width for the head width of the largest host did not alter the results. Means are reported ± SE.

RESULTS

Usurpation attempts by the parasite (observed in 24/31 trials) were characterized by one or more approaches towards the host nest, resulting in physical fighting with the hosts. The initial fight continued until the parasite retreated (11/24 trials), or until the hosts withdrew, leaving the parasite alone on the nest (13/24 trials). Parasites that retreated after the initial fight were observed to make further approaches to the nest in six of 11 trials. Where parasites succeeded in taking over the nest after the initial fight, hosts returned and engaged the parasite in further fighting in eight of 13 trials. At the end of the observation period (1 h after the initial approach), 14 parasites were on the nest and 10 were off the nest.

For those trials in which parasites approached the nest, we examined the influence of the clypeal pattern manipulation on the dynamics of the ensuing fight. Duration of the initial fight was not influenced by the pattern manipulation (LM: paint treatment: $F_{1,23} = 0.35$, $P > 0.5$; treatment*proportion of hosts with clypeal spots: $P > 0.1$; Fig. 2). Independent of the pattern manipulation, fight duration tended to be positively related to the proportion of hosts on a nest with clypeal patterns, although this was not statistically significant ($F_{1,23} = 3.56$, $P = 0.07$). Manipulation of the clypeal pattern also had no effect on the intensity of the first fight, in terms of either the frequency of high-intensity interactions (GLM: $F_{1,23} = 0.31$, $P > 0.5$; treatment*proportion of hosts with clypeal spots: $P > 0.1$) or the proportion of time spent engaged in high-intensity interactions (GLM: $F_{1,23} = 0.04$, $P > 0.5$; treatment*proportion of hosts with clypeal spots: $P > 0.5$). Again, however, there was an independent effect of host clypeal patterns: relatively more time was spent engaged in high-intensity interactions when a higher proportion of hosts had clypeal patterns ($F_{1,23} = 6.55$, $P = 0.02$). The proportion of hosts on a nest with clypeal patterns also tended to be positively associated with the frequency of high-intensity interactions, although this was not statistically significant ($F_{1,23} = 3.60$, $P = 0.07$).

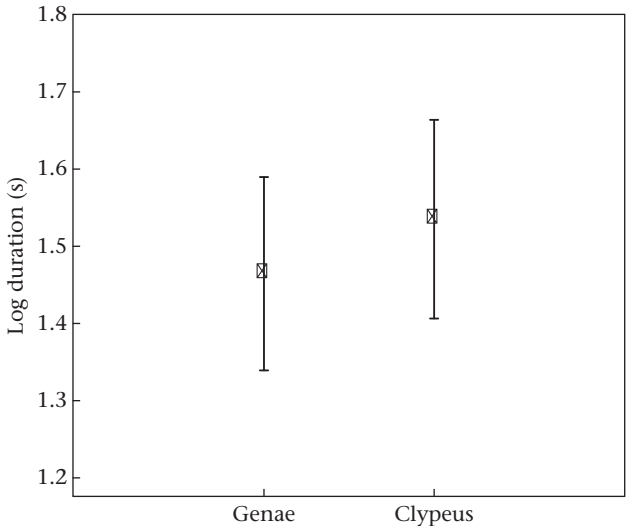

Figure 2. Duration of initial fights in relation to parasite manipulation (paint concealing the genae or clypeus). Points and bars show means ± 1 SE. $N = 24$ fights.

To explore the role of body size in RHP, we compared the sizes of winners and losers. Winners of the first fight (i.e. the fight following the initial approach by the parasite) were not significantly larger than losers (GLMM: $L_1 = 0.86$, $P > 0.1$; winners: 3.57 ± 0.02; losers: 3.54 ± 0.03 mm). However, in a second comparison of winner and loser size, this time based on whether nests had been usurped by the end of the observation period (i.e. 1 h after the first interaction), winners were significantly larger than losers (GLMM: $L_1 = 5.45$, $P = 0.02$; winners: 3.59 ± 0.02; losers: 3.51 ± 0.02 mm; Fig. 3). There was no significant interaction between outcome and species, indicating that the importance of size in determining fight outcome did not vary between species ($L_1 = 2.10$, $P > 0.1$). Two processes could account for the increase in size difference between winners and losers observed over the observation period. First, larger parasites that initially failed to usurp the nest may win against hosts in further fights. Although small sample sizes preclude statistical analysis, a comparison of mean head widths suggests this might be the case: average head width of parasites that subsequently usurped the nest was greater than that of parasites that lost the first fight and did not ultimately manage to usurp nests (3.69 ± 0.06 versus 3.52 ± 0.05 mm, $N = 3$ and 8, respectively). Additionally, or alternatively, smaller parasites may be more vulnerable to eviction from the nest if they are less able to resist further attacks from hosts following initial usurpation.

The importance of body size as a determinant of contest outcome indicates that information about own size and possibly rival size may be useful to individuals when choosing whether to persevere or retreat. To test for possible assessment rules, we

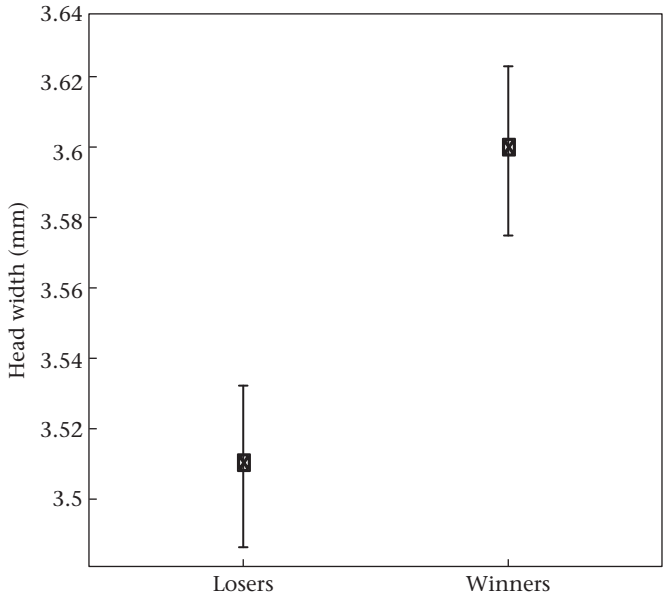

Figure 3. Mean head width of winners and losers, based on whether the parasite was on the nest at the end of the observation period (1 h after first approach). Points and bars show means ± 1 SE. $N = 24$ fights.

analysed the relationships between contest dynamics and the size of winners and losers. Focusing on the first fight, we found no significant relationship between fight duration and the size of winners (LM: $F_{1,23} = 0.00$, $P > 0.5$) or losers ($F_{1,23} = 0.00$, $P > 0.5$). We also found no relationship between the intensity of the first fight and the size of winners or losers, whether considering the frequency (GLM: winners: $F_{1,23} = 0.05$, $P > 0.5$; losers: $F_{1,23} = 0.16$, $P > 0.5$) or duration (GLM: winners: $F_{1,23} = 0.16$, $P > 0.5$; losers: $F_{1,23} = 0.01$, $P > 0.5$) of high-intensity interactions.

Total duration of all fights that occurred within the observation period was not significantly predicted by either winner size (LM: $F_{1,23} = 0.67$, $P > 0.1$) or loser size ($F_{1,23} = 0.20$, $P > 0.5$). Likewise, the total number of fights was not significantly predicted by size of either winners (GLM: $\chi^2_1 = 1.62$, $P > 0.1$) or losers ($\chi^2_1 = 2.27$, $P > 0.1$).

DISCUSSION

The Importance of Clypeal Patterns

We found no evidence that clypeal patterns in the social parasite *P. semenowi* function to minimize aggression from hosts during nest usurpation. Previous research into rival assessment in North American populations of *P. dominulus* has emphasized the importance of clypeal patterns as signals of RHP in settling contests between unfamiliar individuals (Tibbetts & Lindsay 2008; Tibbetts et al. 2010). While evidence for intraspecific status signalling via clypeal patterns in European populations is more limited (Cervo et al. 2008; Zanette & Field 2009; Green & Field 2011), results from a recent experiment have suggested that such patterns may be important in interspecific contests (Ortolani et al. 2010). Presentation experiments using heads of a related social parasite *P. sulcifer* demonstrated an increase in host aggression towards parasite heads that had been manipulated to conceal the clypeal pattern (Ortolani et al. 2010). This finding raises the possibility that parasite clypeal patterns could function as interspecific status badges, exploiting a mechanism of conflict resolution among hosts to dissuade hosts from aggressive retaliation during nest usurpation. Alternatively, the parasite's patterns may function as 'amplifiers' (Taylor et al. 2000), highlighting the width of the mandibles (Ortolani et al. 2010). Here, the clypeal pattern communicates fighting ability by amplifying a trait (mandible width) that is larger in *Polistes* social parasites than in their hosts and thought to confer superior fighting ability (Cervo 2006; Ortolani et al. 2010). In our experiment, which simulated real usurpation contests between *P. dominulus* hosts and the social parasite *P. semenowi*, concealment of the pattern did not result in greater aggression from hosts, in terms of either fight duration or intensity. In addition, a nonsignificant interaction between treatment and the proportion of hosts with clypeal marks showed that this response to the paint treatment was not dependent on hosts' clypeal patterns. Our results therefore suggest that *P. semenowi* clypeal patterns are not used in rival assessment during nest usurpation, either as status badges or amplifiers. The relatively small sample sizes in our study, necessitated by logistical considerations (i.e. the relative rarity of parasites in our population), might suggest that we cannot put too much faith in the negative results we have obtained. The effect size (Cohen's d) that we obtained was 0.24 with 95% confidence interval = −0.56–1.05 (note that this is on a log scale). Thus, for our analysis to detect a significant influence of the manipulation on fight duration, the effect size would need to be large (i.e. >1.05 standard deviations; similar effect sizes would also be necessary to detect a significant effect of the manipulation on fight intensity). However, other experiments using our Spanish population and employing larger sample sizes have also found no evidence that hosts react to rival clypeal patterns, this time during interactions with conspecifics (Green & Field 2011). Below, we discuss the difference in results between this study and that of Ortolani et al. (2010) in terms of the context dependence of receiver behaviour and the costs and benefits of rival assessment during usurpation contests (see Limitations on Rival Assessment).

Although parasite clypeal patterns play no role in usurpation contests, host clypeal patterns do influence fight dynamics: initial fights were significantly more intense and also tended to be longer in trials where a greater proportion of defending hosts had clypeal patterns. One explanation for this is that hosts with clypeal patterns have higher RHP and are better able to resist the invading parasite, investing energy in prolonged and high-intensity defence behaviours (Tibbetts & Dale 2004; Tibbetts & Shorter 2009), although we again note that other experiments on this population have found no evidence that clypeal patterns communicate this information to conspecifics (Green & Field 2011). A second explanation is that parasites are more motivated to attack nests of patterned hosts because such colonies are more attractive to the parasite, leading to longer and more intense fights. Previous work has shown that *Polistes* social parasites preferentially target larger nests with mature brood (Cervo & Turillazzi 1996; Shreeves et al. 2003). If host clypeal patterns reflect aspects of quality associated with greater colony size and productivity (e.g. survival, fecundity), then parasites able to assess host clypeal patterns, or traits correlated with patterning, might be expected to target high-quality hosts preferentially. Further research into the characteristics of host colonies targeted by the parasite is needed to determine which host traits are favoured by *P. semenowi*, as well as how these traits are assessed by the parasite during host selection.

The Importance of Body Size

Our analysis revealed the importance of opponent size on outcome of parasite usurpation attempts. Larger parasites were more likely to usurp nests successfully, while larger hosts were more successful in defending nests. Body size has often been highlighted as a potential determinant of RHP in *Polistes* wasps (e.g.

Turillazzi & Pardi 1977; Cervo et al. 2008). In particular, previous studies have indicated that body size influences both intraspecific (Tibbetts & Shorter 2009) and interspecific (Ortolani & Cervo 2010) usurpation contests. Ortolani & Cervo (2010) examined geographical variation in *P. dominulus* body size in relation to parasitism by a second social parasite, *P. sulcifer*. They found that larger hosts were more active in fighting a usurping parasite off the nest, and were less likely to be parasitized (Ortolani & Cervo 2010). Furthermore, the occurrence of larger hosts in populations experiencing high rates of parasitism led them to suggest that larger host body size may be the result of a parasite–host arms race, in which large body size is selected as a defence against parasite invasion (Ortolani & Cervo 2010). Our result suggests that a similar relationship may be found between *P. dominulus* size and *P. semenowi* density; however, more data on host size and parasitism rates from different areas are needed to test this hypothesis.

Our finding that size influences the outcome of usurpation contests raises the possibility that information about opponent size is used in decision making during competitive interactions (Arnott & Elwood 2009). To investigate whether size-based assessment occurs during usurpation contests, we analysed the relationship between duration and intensity of the first fight, as well as the total number and duration of fights within the observation period, and the size of winners and losers (as judged by usurpation success/failure). Looking within the first fight, we found that neither duration nor intensity was significantly predicted by the size of winners or losers. Furthermore, within the whole observation period, neither total fight duration nor the total number of fights was significantly predicted by either loser or winner size. Again, however, we note the relatively small sample sizes available in our study. Calculation of effect sizes indicates that moderate to large effects of size on fight measures could be detected with our sample size. For example, the size of the effect of loser size on contest duration (partial correlation coefficient, r) is -0.1 with 95% confidence interval $= -0.48–0.32$ (on a log scale), and the other effects were of similar sizes.

The absence of a positive correlation between loser size and measures of fight duration and/or intensity in particular argues against assessment by rivals during usurpation. A common prediction of all models of assessment is that contest duration and/or intensity should increase with increasing loser RHP (Arnott & Elwood 2009). This is true for strategies of self-assessment, where the decision to withdraw is taken when some cost threshold, determined by individual RHP, is exceeded (Taylor & Elwood 2003). In the case of mutual assessment, a positive relationship between loser RHP and duration and/or intensity is also expected, given that the decision to withdraw versus escalate is based on RHP asymmetry between contestants, which is most easily perceived when differences in RHP are large (Taylor & Elwood 2003). The absence of a positive relationship between loser size and duration or intensity in this study would therefore appear to rule out assessment during usurpation contests. Thus, while larger body size confers greater RHP, there is no evidence that size assessment is used to minimize fight costs during usurpation attempts.

Limitations on Rival Assessment

The ability to use information about opponent RHP to make tactical decisions during fights is often considered advantageous as it allows individuals to avoid the costs (e.g. injury, depletion of energy reserves) of escalated conflict. Given the benefits of assessment, our finding that wasps do not use information about rival RHP is somewhat surprising. A possible explanation for the apparent lack of assessment is that both hosts and parasite are highly motivated to fight on account of the high value of the contested resource. From the hosts' perspective, usurpation of a colony by a social parasite represents a potentially fatal assault on host fitness. Upon usurpation, the parasite destroys younger brood, preserving older brood as a workforce to rear its offspring (Cervo 2006). Combined with high reproductive skew in favour of the parasite (J. P. Green & J. Field, unpublished data), this often results in a dramatic reduction in host fitness following parasitism. Although it has been shown that *Polistes* foundresses can build new nests following nest predation (Strassmann et al. 1988), the extent to which this is a viable option for parasitized hosts is unknown. Usurpation by social parasites occurs at a relatively late stage in the season (i.e. just prior to worker emergence); opportunities for renesting may therefore be time limited. However, the potential for renesting is likely to influence perceived nest value during host–parasite contests and therefore merits further study, particularly as there is evidence that hosts do occasionally abandon nests following successful usurpation by parasites (E. Almond & J. Field, unpublished data).

From the perspective of the parasite, which is dependent on a host workforce for offspring production, successful usurpation is critical to parasite fitness. Furthermore, parasites enjoy only a brief window in which to attack, in the late pre-emergence period of the host nest cycle (Cervo 2006), which would be expected to limit the number of usurpation attempts a parasite can make, whether on the same nest or on different nests. No direct evidence for multiple usurpation attempts by *P. semenowi* in the wild is available at present, and would require both tracking of individual parasites and detection of all usurpation attempts, which are sometimes very brief (J. P. Green, personal observations).

In the case of usurpation contests, therefore, the fitness payoff associated with successfully usurping or successfully defending the nest may in fact exceed any costs associated with escalated fighting (Enquist & Leimar 1990), thereby negating any benefit of rival assessment. Furthermore, given the enhanced weaponry of *Polistes* social parasites (Cervo 2006), any respect shown for RHP asymmetries during usurpation fights would often, if not always, result in acceptance of the parasite by the host colony, and the fitness costs associated with parasitism. In the face of these 'divisive' asymmetries (Grafen 1987), the optimal strategy may be to persevere irrespective of asymmetries in RHP until the costs of injuries and depletion of energy reserves force retreat (Grafen 1987). Support for this scenario comes from observations (this study) of serious injuries sustained to both hosts and parasites during trials, including the loss of legs and damage to wings. Although such injuries impose significant costs, escalated fighting of the kind we observed may still be favoured if such costs are outweighed by the cost of losing the nest (Enquist & Leimar 1990; Elias et al. 2010).

High resource value may also explain why we found no evidence of rival assessment based on clypeal patterns. The use of signals or cues of RHP is argued to facilitate assessment prior to fighting, thereby minimizing costs of conflict (Maynard Smith & Harper 2003). However, such signals may have limited value in contexts where resource value is high (Maynard Smith & Harper 1988; Tibbetts 2008). As discussed, nest usurpation may be one context in which the value of the resource actually exceeds the cost of fighting; status signals may therefore be of limited importance in such contests. In the experiments by Ortolani et al. (2010), which demonstrated an effect of parasite clypeal patterns on host aggression, receiver responses were not tested in the context of nest usurpation. In such a context, we have found that parasite clypeal patterns do not modulate host aggressive behaviour. In a separate observational study of intraspecific nest usurpation in *P. dominulus*, usurpation success was predicted by the relative clypeal patterns of the intruder and resident (Tibbetts & Shorter

2009). However, signal manipulation experiments testing whether patterns alone determined contest outcome were not performed. If, in real usurpation attempts, the motivation for intruders (conspecifics or social parasites) to attack and for hosts to defend is high, then selection may favour escalation over information gathering that allows tactical retreat based on opponent assessment (Grafen 1987; Enquist & Leimar 1990; Moore et al. 2008).

This study aimed to investigate potential assessment rules guiding interspecific contests between a usurping social parasite and its hosts. We found no evidence that parasite clypeal patterns function to reduce host aggression during contests. Larger parasites were more successful in usurping nests, and larger hosts more successful in defence, but assessment based on size does not appear to occur during contests. We focused on size as a determinant of RHP in this study; future work that investigates assessment based on other RHP determinants would provide a clearer picture of assessment during *Polistes* host–social parasite contests. Studies that explored the physiological basis of energetic costs during usurpation fights (Weiner et al. 2009) may be particular valuable in this regard.

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Interpopulation variation in status signalling in the paper wasp *Polistes dominulus*

Jonathan P. Green*, Jeremy Field

School of Life Sciences, University of Sussex

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Contests between individuals over resources may be costly in terms of both time and energy expended and the risk of injury. Signals of status, or 'status badges', are thought to have evolved to minimize these costs by providing information about an individual's fighting ability or resource-holding potential (RHP) at the start of a contest. Studies on recently established North American populations of the paper wasp *Polistes dominulus* have demonstrated the existence of a status badge, in the form of black clypeal patterns, and have shown that rivals attend to these patterns during competitive interactions. However, observational data from studies in this wasp's native European range have failed to demonstrate a strong link between clypeal patterning and RHP. We undertook the first direct test of status signalling in a European population of *P. dominulus*, by testing receiver responses to clypeal pattern manipulations in a competitive foraging context. We found no evidence that individuals assessed rivals using the clypeal 'badge'. We discuss possible reasons for variation in signal use between the American and European populations, including genetic drift and environmental effects of the development and transmission of the signal.

Contests between individuals over resources can inflict significant costs on both contestants, in terms of time, energy and the risk of injury or death. Theory suggests that the costs of escalated conflicts will be minimized if individuals can assess the competitive ability of their opponents at the start of contests, and thereby predict the likely outcome of a fight (Maynard Smith & Harper 1988). Conventional signals (otherwise known as status signals, or 'status badges') have been argued to play a role in resolving contests over relatively low-value resources, where the fitness payoffs of acquiring the resource are less than the fitness costs of escalated fighting (Rohwer 1975; Maynard Smith & Harper 1988). Visual status signals are small patches of colour that convey information about an individual's competitive ability, or resource-holding potential (RHP), to an opponent. These signals are 'conventional' in the sense that they are not causally linked to RHP, and are furthermore considered to be relatively cost free to produce, in contrast to indices and strategic signals (Maynard Smith & Harper 2003).

Status signals have attracted much controversy on both theoretical and empirical grounds over the issue of how signal reliability is maintained (reviewed in e.g. Senar 1999; Whiting et al. 2003). Explanations for the reliability of cheap, arbitrary status signals have largely focused on costs incurred during social interactions, which act to prevent cheating (Senar 1999; Tibbetts & Dale 2004). Scenarios for these 'social costs' rely on the fact that escalated fighting is most likely between rivals each signalling at a high intensity (Whiting et al. 2003). For example, a dishonest signaller that attempted to extricate itself from an escalated conflict might be punished if an opponent detected the incongruence between its advertised status and its submissive behaviour (Rohwer 1977; Senar 1999).

Empirical support for status signalling has come largely from studies of passerines (e.g. Qvarnström 1997; Senar & Camerino 1998) and lizards (Whiting et al. 2003). Recently, however, a number of studies suggest that status signalling also operates in the paper wasp *Polistes dominulus* (Tibbetts & Dale 2004; Tibbetts & Lindsay 2008; Tibbetts et al. 2010). *Polistes dominulus* is a primitively eusocial species which lacks morphological castes. It has a cosmopolitan distribution, following recent expansions from its native Eurasian range into North and South America and Australia (Cervo et al. 2000). Nests are founded in the spring, either by lone foundresses or by groups of cofoundresses, all of which are inseminated and can potentially lay eggs. In cofoundress groups, egg laying is determined by rank within a linear hierarchy that emerges during nest founding, with dominant (Rank 1) females monopolizing reproduction. In North American populations, patterns of melanin on the clypeus are argued to signal status in both dominance (Tibbetts & Dale 2004) and competitive interactions (Tibbetts & Lindsay 2008; Tibbetts et al. 2010). The active signal component, 'brokenness' (a measure of pattern disruption; Tibbetts 2010), has been shown to be a reliable signal of RHP, with social costs enforcing honesty (Tibbetts & Dale

* Correspondence: J. P. Green, School of Life Sciences, JMS Building, University of Sussex, Falmer, Brighton BN1 9QG, U.K.
E-mail address: j.p.green@sussex.ac.uk (J.P. Green).

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2004). However, two studies in the species' native Eurasian range have failed to find evidence of a strong correlation between pattern brokenness and hierarchical rank in spring foundress groups (Cervo et al. 2008; Zanette & Field 2009). Cervo et al. (2008) were also unable to demonstrate a link between clypeal patterning and survival and health. These findings cast doubt on the function of clypeal patterns as badges of status in European populations and indicate that there may be geographical variation in status signalling in *P. dominulus*. However, support for this hypothesis requires a manipulative test of status signal use in Europe, which to date has been lacking.

In this study, we tested for status signalling in the Spanish population of *P. dominulus* studied by Zanette & Field (2009). In testing for a signal function in the clypeal pattern, we followed the experimental procedure of Tibbetts & Lindsay (2008). The demonstration of status signalling requires that manipulation of the putative signal should result in a change in behaviour in the receiver, consistent with the idea that the signal conveys information about individual RHP. This is not straightforward, for two reasons (Senar 1999). First, a change in receiver behaviour may be caused by difficulties with individual recognition following manipulation of the signaller (Shields 1977). Second, studies using familiar individuals run the risk of confounding the demonstration of status signalling with the demonstration of anticheating mechanisms, which may interfere with receiver responses if the signaller's true status is known to the receiver (Senar 1999). It is important, therefore, that the receiver has not interacted with the signaller prior to the manipulation, and that the receiver possesses no information about the signaller's RHP, other than what is signalled. The design used here and by Tibbetts & Lindsay (2008) fulfils these requirements: focal wasps were presented with a choice of two food sources, each guarded by a potential rival with artificially enhanced or reduced clypeal patterns. These 'guards' had not previously interacted with the focal wasps. Furthermore, the guards were presented dead to prevent focal wasps detecting any incongruence between behaviour and advertised status. If wasps do use clypeal patterns to assess rivals in a contest situation, we expected to see focal wasps avoiding the high-status guard, and instead preferring to challenge the low-status guard for access to food, as seen in Tibbetts & Lindsay (2008).

METHODS

Protocols followed Tibbetts & Lindsay (2008). Wasps were collected in early May 2010 at sites around Conil de la Frontera (Cádiz Province, Spain). All wasps were spring foundresses from different nests in the pre-emergence phase of the colony cycle. Guards and focal wasps were collected from sites at least 3 km away to minimize the likelihood of previous interaction. Wasps were transferred to large plastic containers (25 × 16 cm and 16 cm high) where they were held for 48 h, and provided with ad libitum water and sugar cubes. Focal wasps and guards were housed in separate containers. Wasps were then placed individually in plastic tubes and starved for 24 h prior to trials.

Trials were carried out in a rectangular arena (6 cm wide × 7 cm long) with a convex semicircle at one end. At the other end, a sugar cube with a freeze-killed 'guard' wasp on top was placed in each corner. Guards were matched by size (within 0.1 mm wing length) and by the number of clypeal spots; care was taken to select guards of intermediate wing length (range 10.39–12.39 mm), thereby avoiding very large or very small guards. The active signalling component of the pattern is argued to be the amount of disruption, or 'brokenness' (Tibbetts 2010); however, when manipulating patterns, previous studies have altered the number of facial spots, as this is a good proxy for brokenness (Tibbetts & Lindsay 2008). One guard of each pair was painted with Testor's enamel paint to signal a higher status while the other guard was painted to signal a lower status. We used three combinations of guard facial patterns. Wasps with no spots were painted to produce guards with one and two spots, respectively. Wasps with one spot were painted to produce guards with no and two spots, and wasps with two spots were painted to produce guards with no and one spot. A total of 13 individual pairs of guards were used in 149 trials.

Focal wasps were placed within a covered tube at the semicircular end of the arena for 5 min prior to the trial. Wasps were then released into the arena and their choice of sugar cube recorded. We considered the wasp had made a choice when it approached a sugar cube and began to eat. Wasps were given 30 min to make a choice; the median time from release until eating was 50 s (range 2 s–28 min). Fresh sugar cubes were added at the start of each trial.

Previous research has suggested that, when choosing between guards, wasps use information about their own RHP in addition to information about guard RHP (Tibbetts et al. 2010). For example, focal wasps with more clypeal spots showed no preference when presented with guards with no or one spot (Tibbetts et al. 2010). To control for potential effects of focal wasp quality on guard choice, all focal wasps used in the trials had the same number of clypeal spots (i.e. none). We chose to use no-spot individuals as focal wasps in the trials as they represent the most frequent clypeal pattern type in our population (see Discussion), thus permitting a large sample size in the experiment.

Statistics

All analyses were performed using the software R version 2.9.2 (http://www.r-project.org/). We tested whether wasps preferred to challenge the high-spot or low-spot guards using a Pearson chi-square test. To test the effect of specific pairings of guard facial patterns (i.e. no versus one spot, no versus two spots and one versus two spots) on wasps' choices, we used a generalized linear mixed model with binomial errors. Wasp choice was the binary dependent variable (0 = low-spot guard; 1 = high-spot guard) and guard facial pattern pair was the explanatory variable. Although guards were matched for size, we included guard size as a second explanatory variable to test whether small differences in size influenced guard choice. Whether the chosen guard was in the left or right corner of the arena was also included as an explanatory variable. Finally, individual guard pair was added to the model as a random effect to control for similarities within guard pairs in terms of focal wasp choice. Model simplification proceeded by backwards deletion of nonsignificant terms until further removals led to significant ($P < 0.05$) increases in deviance. Significance levels are reported on the addition of nonsignificant terms, and removal of significant terms, from the minimal adequate model.

RESULTS

Our main finding was that focal wasps did not challenge the low-spot guard significantly more often than the high-spot guard (Pearson chi-square test: $\chi^2_2 = 1.71$, $P = 0.43$; Table 1). Furthermore, the same result was obtained when we considered only trials in which focal wasps chose between no- and one-spot guards or

Table 1
Numbers of focal wasps choosing to challenge high-spot and low-spot guards

Guard facial pattern (no. of spots)	Low-spot guard	High-spot guard
0 versus 1	4	4
0 versus 2	18	14
1 versus 2	19	27
Total	41	45

between no- and two-spot guards (Pearson chi-square test with Yate's correction: $\chi^2_2 = 0.01$, $P = 0.94$). This indicates that the lack of preference for the low-spot guard was not the result of a specific failure to choose between one- and two-spot guards, which might be expected if mutual assessment is occurring (i.e. no-spot focal wasps might not be motivated to choose between one- and two-spot guards, if both represent superior competitors). Rather, our results demonstrate a general failure to choose between guard facial patterns, irrespective of the specific combination of guards.

Analysis of factors influencing the focal wasp's choice of the low-spot or high-spot guard showed no influence of guard position in the arena on focal wasp choice ($\chi^2_1 = 1.15$, $P = 0.28$). The analysis, however, revealed a significant interaction between the guard facial pattern pairing and guard size ($\chi^2_2 = 31.01$, $P < 0.001$). When presented with guard pairs with either no and one spot or no and two spots, wasps that chose the smaller guard were more likely to choose the guard with more spots. However, this tendency was not observed among wasps choosing between guard pairs with one and two spots.

To analyse the effects of guard facial pattern and size on focal wasp choice in more detail, we ran a second model in which the interaction was omitted. In the absence of the interaction, neither guard facial pattern pairing nor size had a significant effect on focal wasp choice (facial pattern pair: $\chi^2_2 = 1.52$, $P = 0.47$; size: $\chi^2_1 = 0.08$, $P = 0.77$). Thus, when all guard pair types are considered, focal wasps showed no preference for either the smaller or larger guard. Similarly, when guard size is not considered, the focal wasps' choice of the low-spot or high-spot guard did not depend on the specific combination of facial patterns presented by the guard pair.

During the trials, 63 focal wasps did not eat at either sugar cube. To test whether the likelihood of eating (i.e. the likelihood of approaching either guard) was influenced by the particular combination of guard patterns, a second generalized linear mixed model was run. Decision to eat was the binary dependent variable, and guard facial pattern pairing and guard size were the independent variables. Guard size was taken as the size of the smallest guard in a pair. Individual guard pair was again added as a random effect. The analysis revealed no significant effect of either guard facial pattern pairing or guard size on the decision to eat (guard facial pattern: $\chi^2_2 = 0.69$, $P = 0.41$; guard size: $\chi^2_1 = 0.05$, $P = 0.83$). Furthermore, the interaction between guard facial pattern and size did not influence the probability of eating ($\chi^2_2 = 0.34$, $P = 0.57$). Therefore, the decision to approach either guard and eat the sugar was not influenced by the relative size of the guards or by the particular combinations of guard clypeal patterns (i.e. wasps were equally likely to eat when presented with pairs of guards with no versus one spot, no versus two spots or one versus two spots).

These results show that focal wasps did not prefer to challenge the low-spot guard. To exclude the possibility that this result is due to differences in experimental set-up (e.g. illumination), we conducted another set of trials in which wasps were given the choice between a sugar cube guarded by a no-spot wasp and an unguarded cube. If wasps can perceive the presence of a guard, then wasps should prefer to feed at the unguarded sugar cube to avoid competition. We found that wasps were significantly more likely to feed at the unguarded sugar cube (binomial test: $N = 35$, 69%, $P < 0.05$). This result is important as it demonstrates that wasps perceive the presence of another wasp in our set-up, and so could use information about the guard's phenotype when choosing between guards.

DISCUSSION

We found no evidence that wasps assess conspecifics via visual status signals in a foraging context. When given the choice of two food sources, one guarded by a high-spot guard and the other by a low-spot guard, focal wasps did not show a preference for feeding near the low-spot guard. This suggests that the decision to challenge a particular rival is made without reference to the rival's clypeal patterns, in particular the number of clypeal spots. We also found that the decision to approach either guard (i.e. the decision to eat) was not influenced by particular combinations of guard facial patterns. We observed a significant interaction between guard size and the guard facial pattern type, although this is difficult to interpret in light of previous work. The finding that individuals choosing high-spot guards from certain pairings (no versus one spot or no versus two spots) tended also to choose the smaller guard could be seen as evidence for rival assessment, with individuals attempting to minimize competition by choosing opponents of smaller size when facial patterns indicate high status. However, it is not clear why these effects are seen only in these pairs of guard facial patterns, and not in the choice trials involving one- and two-spot guards. In the absence of any interaction, the specific combination of guard facial pattern did not influence focal wasp choice, consistent with the idea that relative differences in signal intensity (i.e. brokenness, or number of spots), rather than specific patterns, are important to the receiver when assessing rivals (Tibbetts & Lindsay 2008).

Our finding that clypeal patterns are not involved in rival assessment contrasts with that of Tibbetts & Lindsay (2008), who have found evidence for status signalling in a North American population of the same species. In the same set-up as used in the present study, Tibbetts & Lindsay (2008) demonstrated a strong preference for feeding near the low-spot guard. Preference for the low-spot guard was observed regardless of the specific combinations of guard facial patterns, suggesting that focal wasps discriminated between rivals based on facial patterns in general, rather than showing preference for, or avoidance of, a particular pattern (Tibbetts & Lindsay 2008). Furthermore, in a second choice experiment, Tibbetts et al. (2010) again demonstrated a preference for feeding near the low-spot guard, although this preference was dependent on the facial pattern of the focal wasp (Tibbetts et al. 2010). Taken together, these findings suggest that, in North American populations of *P. dominulus*, foundresses attend to clypeal patterns during social interactions and behavioural responses are determined, at least in part, by asymmetries in clypeal patterning between rivals.

This study represents the first explicit test of status signalling in a population of *P. dominulus* in its native European range. Given the number of studies furnishing empirical support for status signalling in *P. dominulus* (Tibbetts & Dale 2004; Tibbetts 2008; Tibbetts & Lindsay 2008; Tibbetts & Shorter 2009; Tibbetts et al. 2010), it is perhaps surprising that we failed to find evidence for status signals operating in our population. As we note above, the experimental set-up used for the choice trials closely resembles that used in previous studies. One difference, however, is the use of no-spot focal wasps, rather than the one-spot wasps used by Tibbetts & Lindsay (2008). No-spot wasps were used to maximize our sample size, as they represent the most common pattern type in our population (Zanette 2007). However, a potential concern with using no-spot wasps rather than one-spot wasps is that the two groups may behave differently if information about personal quality is also used in guard choice (Tibbetts et al. 2010). A previous study by Tibbetts et al. (2010) found that no-spot wasps showed no preference for one- versus two-spot guards: no-spot guards are considered to be of low quality, and are argued not to choose between opponents of higher quality (one- and two-spot guards). Although the no-spot focal wasps used in our experiment may not have discriminated between one- and two-spot guards for this reason, no-spot wasps using mutual assessment would still be expected to challenge the no-spot guard more often in the no- versus one-spot and no versus two-spot guard combinations. This is because a no-spot guard represents a more equal competitor (i.e. signals a similar RHP). Our finding that focal wasps do not prefer

to challenge the no-spot guard when present indicates that the lack of preference is not the result of strategic decisions using information on individual quality, but rather is consistent with the absence of visual status signalling in this population.

The level of motivation experienced by the focal wasps is a second factor that may account for the difference between the results from this study and those from previous studies. Tibbetts (2008) has shown that when hunger levels are increased, thereby increasing the value of the food source, wasps are more likely to challenge a high-spot guard. Could high hunger levels of focal wasps account for the absence of rival assessment in this study? Several aspects of our experimental design suggest that this is unlikely. First, focal wasps used in our experiment were starved for 24 h prior to the choice trials, the same starvation period experienced by wasps in Tibbetts & Lindsay's (2008) study. Prior to this period, wasps were provided with ad libitum sugar for 48 h: food reserves were therefore unlikely to be low before the starvation period. Although Tibbetts (2008) found that motivation to challenge a guard increased with increasing hunger levels, this effect was only observed when comparing wasps starved for 3 days and wasps given unrestricted food. Wasps starved for 24 h were not significantly more likely to approach the high-spot guard than wasps that had not been starved; they were not significantly quicker to approach the food source and did not spend longer eating (Tibbetts 2008). Although Tibbetts (2008) used one-spot focal wasps, there is currently no reason to believe that tolerance to starvation varies systematically with clypeal patterning, such that adults with more elaborate clypeal patterns are better able to withstand starvation. Indeed, the fact that, in our study, latency to eating was similar to that reported previously (median time to eating was 50 s versus 1 min in Tibbetts & Lindsay 2008) suggests that focal wasps were similarly motivated when presented with the choice task. The fact that a significant proportion of wasps tested did not approach the sugar after the starvation period also indicates that hunger levels in focal wasps were not too high to preclude rival assessment.

Finally, a third possible reason for the absence of a preference between guard facial patterns in this experiment may have been that the focal wasps were unable to see the guards. However, in a second experiment, wasps showed a significant preference for eating at the unguarded versus guarded sugar cube. This result indicates wasps were able to see guards and supports the assumption underlying the choice paradigm that wasps should prefer to feed wherever the risk of competition from conspecifics is lowest. Overall, we feel confident that our result is not an artefact arising from differences in experimental design. This points to a genuine difference in signal function between American and European populations: clypeal patterns do not appear to represent 'badges of status' in Spanish *P. dominulus*.

Our negative result is consistent with other observations on European populations of *P. dominulus* (Cervo et al. 2008; Zanette & Field 2009). Observing dominance interactions between cofoundresses on established nests, Cervo et al. (2008) found that neither the disruption ('brokenness') nor the size of the pattern differed significantly between Rank 1 and Rank 2. In a separate study, Zanette & Field (2009) explored the influence of clypeal patterns on social rank earlier in the pre-emergence phase of the colony cycle, before group composition had stabilized. Within cofoundress associations, the size of the clypeal pattern had a weakly significant effect on rank, with Rank 1 sporting a larger black mark than her subordinates. Although cited as evidence for an association between clypeal patterning and dominance (Tibbetts & Shorter 2009), Zanette & Field's (2009) results should be interpreted with caution. First, the effect of clypeal patterning was no longer significant when other potential determinants of rank were included in the regression (Zanette & Field 2009). Second, the study measured only the size of the clypeal mark, and did not quantify pattern disruption (brokenness). Tibbetts (2010) has argued that the area of the pattern represents the nonsignalling component, while the active signalling component is the pattern's brokenness. An (weak) association between pattern size and rank would therefore say little about the potential for status signalling in the Spanish population. Indeed, among patterned wasps in this population, area and brokenness are not significantly correlated (J. P. Green & J. Field, unpublished data).

A striking feature of the Mediterranean populations is that clypeal pattern variability is much lower than has been reported for American populations. While 85% of wasps in the population investigated by Tibbetts & Dale (2004) show some clypeal patterning, only 40% of wasps in Italy (Cervo et al. 2008) and as few as 15% in Spain (Zanette 2007) have these patterns. An important consequence of this low variability in clypeal patterning is that its importance in mediating social conflict is necessarily limited. The presence of clypeal patterning is neither necessary nor sufficient for the acquisition of high rank, as demonstrated by Cervo et al. (2008) and Zanette & Field (2009). Furthermore, the majority of contests over resources such as food or reproduction will be between individuals without clypeal marks. Rival assessment based on clypeal patterning will therefore often fail to reveal differences in individual RHP in these populations. We suggest that the difference in prevalence of clypeal patterns between Mediterranean and American populations can be considered further evidence of genuine variation in signal use between these populations.

Geographical variation in the value of secondary sexual traits is well documented (Wilczynski & Ryan 1999; Dunn et al. 2008; Takahashi et al. 2008). However, most studies have focused on the value of intersexual traits (i.e. 'ornaments' used in mate choice), while there are few accounts of geographical variation in the value of intrasexual traits, including conventional (status) signals. One possible example of population divergence in status signalling comes from studies on the collared flycatcher, *Ficedula albicollis*, in Sweden (Pärt & Qvarnström 1997; Qvarnström 1997) and Hungary (Garamszegi et al. 2006). In Sweden, the forehead patch acts as a signal of status in territorial contests between males (Pärt & Qvarnström 1997; Qvarnström 1997), whereas in Hungary, territory defence is not associated with forehead patch size (Garamszegi et al. 2006). The reason for this variation in plumage function is not currently understood, but in *F. albicollis* there is some evidence that the forehead patch is also a target of female choice (Gustafsson et al. 1995). In cases where status signals are additionally used in mate choice, variation in the signal phenotype could be explained by divergence in female preference for the trait, rather than changes in the intensity of intrasexual competition between populations.

In *P. dominulus* wasps, clypeal patterns are present only on females, and there is no evidence from observations of mating behaviour that males choose among females (Beani 1996). What then might account for variation in the frequency of clypeal patterns and their use as signals between populations? One factor promoting variation may be genetic drift, with populations passing through genetic bottlenecks during the first founding events in the U.S.A. 30–40 years ago (Cervo et al. 2000). There is evidence that the size of the clypeal mark is a heritable trait (Tibbetts 2010), suggesting that an initial difference in pattern prevalence may be preserved or even amplified over generations. However, the recent finding of relatively high genetic variability in American populations points to multiple independent founding events (Liebert et al. 2006), suggesting that bottlenecks may not have been particularly severe.

An alternative explanation could be that variation in pattern prevalence and function is due to environmental effects on the development of the clypeal pattern. Under the developmental conditions present in some environments, clypeal patterning may be prevalent/variable enough within the population to function as

a status badge, while conditions in other environments result in a less variable pattern. In particular, there is good evidence that climatic factors, such as temperature and humidity, affect the extent of melanin patterning in insects (Usui et al. 2004; Parkash et al. 2008), including *Polistes* wasps (Enteman 1904). Climate differences could therefore potentially explain the interpopulation variation in clypeal patterning in *P. dominulus*, with lower temperatures and/or higher humidity generating more variable clypeal patterns in populations at higher latitudes, as is seen in a number of bird species (Price 2006). This hypothesis is consistent with the greater pattern variability in New York State than in our southern Spanish population. Data on pattern variability and status signalling from other, more northerly, populations in the species' ancestral range would provide a clearer picture of possible climatic effects on pattern development and signal function.

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Unrelated Helpers in a Primitively Eusocial Wasp: Is Helping Tailored Towards Direct Fitness?

Ellouise Leadbeater[1]*, Jonathan M. Carruthers[1,2], Jonathan P. Green[1], Jasper van Heusden[1], Jeremy Field[1]

1 School of Life Sciences, University of Sussex, Brighton, United Kingdom, **2** Natural Environment Research Council (NERC) Biomolecular Analysis Facility, University of Sheffield, Sheffield, United Kingdom

Abstract

The paper wasp *Polistes dominulus* is unique among the social insects in that nearly one-third of co-foundresses are completely unrelated to the dominant individual whose offspring they help to rear and yet reproductive skew is high. These unrelated subordinates stand to gain direct fitness through nest inheritance, raising the question of whether their behaviour is adaptively tailored towards maximizing inheritance prospects. Unusually, in this species, a wealth of theory and empirical data allows us to predict how unrelated subordinates should behave. Based on these predictions, here we compare helping in subordinates that are unrelated or related to the dominant wasp across an extensive range of field-based behavioural contexts. We find no differences in foraging effort, defense behaviour, aggression or inheritance rank between unrelated helpers and their related counterparts. Our study provides no evidence, across a number of behavioural scenarios, that the behaviour of unrelated subordinates is adaptively modified to promote direct fitness interests.

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* E-mail: e.leadbeater@sussex.ac.uk

Introduction

Nests of the primitively eusocial paper wasp *Polistes dominulus* are founded in the spring by one or a small group of overwintered reproductive females. In groups, one female becomes dominant and monopolizes reproduction, while subordinates forage to feed the brood [1,2]. These co-foundress associations are unique amongst social insect breeding groups, because many subordinate wasps are completely unrelated to the dominant, but lay almost none of the eggs [3,4,5]. Given that foundresses survive for only one breeding season, breeding independently would seem to represent a better option than helping an unrelated wasp to breed, but the potential benefits of group membership become apparent when the possibility of nest inheritance is considered [6]. Foundresses live in small groups and queen mortality rates are high, so subordinates may have a significant chance of inheriting the dominant position [4,7,8,9]. Thus, even though subordinates may obtain no *current* direct fitness while the dominant is alive, they have potential "future fitness" [9]. Whilst this might help to explain why unrelated subordinates join nests, it does not explain why they devote time and energy to brood care. Why should individuals forage for and protect brood in which they have no kin-selected interest, rather than simply waiting to inherit?

One possible explanation for helping by unrelated subordinates is that wasps cannot accurately discriminate kinship at the individual level. However, at least some *P. dominulus* co-foundresses must derive from different natal nests, because relatedness is typically lower in co-foundress associations than within broods on the previous season's nests from which they derive [4]. Inter-nest (*cf* intra-nest) kin discrimination is common in wasps [10], suggesting that at least some individuals should be recognized as non-kin. Furthermore, there is evidence that the chemical information necessary for discrimination even between different sister groups born on the same natal nest is present in this species [11]. Thus, it seems likely that unrelated subordinates may indeed recognize that they are not kin of the wasp whose offspring they help to rear.

In this paper, we test the hypothesis that the helping behaviour of non-relatives is tailored towards attaining future fitness. In other words, unrelated subordinates may choose to avoid participating in tasks that might compromise their chances of inheritance. The *P. dominulus* study system is unusual in that extensive previous work allows us to identify such contexts. For example, in many species, it might be difficult to predict whether foraging for unrelated brood will improve or worsen an individual's prospects of gaining future fitness through inheritance. On the one hand, rearing brood will increase group size, providing a larger workforce should inheritance occur, and possibly also boosting the helper's own chance of survival [12,13,14]. On the other, the energetic costs or mortality risks of helping might be substantial. In primitively eusocial wasps, however, prior work has found that the costs of foraging to future fitness most likely outweigh the benefits. In both *P. dominulus* [15] and *Liostenogaster flavolineata* [9], another small-group social wasp where inheritance is common, subordinates reduce their foraging effort when they attain higher ranks in the queue to inherit the nest. This supports theoretical findings [15,16] that when future fitness is a realistic possibility, as it is for higher-ranked wasps, the high mortality risk of foraging leads to selection for reduced helping effort.

On this basis, we predict that since unrelated subordinates are under selection to maximize future fitness, they will forage less than their related counterparts. Queller and colleagues [4] have previously found a marginally significant tendency for more distant relatives of the dominant to spend less time foraging, in a small sample under laboratory conditions. Empirical data from *P. dominulus* also allow us to predict how unrelated subordinates should behave towards their nestmates. Cant and Field [17] found that wasps that were highly-ranked in the queue to inherit were more aggressive towards their nestmates, whilst Cant and colleagues [18] showed that Rank 2 wasps that stood to inherit larger groups were more likely to escalate experimentally-induced conflicts with the dominant. Both results are consistent with theoretical predictions that selection to maximize future fitness promotes aggression towards nestmates, especially the dominant wasp [18,19], perhaps because aggressive behaviour might improve a wasp's position in the queue to inherit the nest [18].

In this study, we add two further behavioural contexts where we consider that helping might compromise future fitness. We investigate nest defense behaviour, because defending the nest from conspecific usurpers carries a risk of serious injury [20]. If unrelated subordinates can choose their own level of effort, they might be less willing to contribute to group nest defense. We also investigate whether unrelated subordinates occupy higher ranks in the queue to inherit the nest. In summary, we test the hypotheses that foraging effort, intra-nest aggression, nest defense behaviour, and inheritance rank, will vary according to a subordinate's relatedness to the dominant.

Parallels with the *P. dominulus* social system may be found amongst certain inheritance-based vertebrate co-operative groups, where attempts to link helping effort to relatedness have produced mixed results [21,22,23,24]. Our invertebrate study system is unusual in that a wealth of theoretical and empirical work has demonstrated the effects of variation in future fitness on helping behaviour, allowing predictions about how unrelated subordinates should behave. In the following experiments, we make use of this opportunity to compare the helping behaviour of individuals to which kin-selected benefits can and cannot apply.

Methods

Behavioural studies

In early March 2009, we selected 241 nests on hedges of *Opuntia* cactus running along the edges of a mixed arable/pasture site in Southern Spain. We individually marked (Humbrol paints) and clipped a tarsal sample (stored in 1 ml pure ethanol) from each foundress. For six weeks starting 1st April, we selected groups of approximately six marked nests each day, each with 3–6 foundresses and large larvae or older brood. The sequence of experiments is summarized in Figure 1.

Work effort and inheritance rank. To ascertain whether unrelated subordinates forage relatively less, we visited each nest approximately every 45 minutes on sunny afternoons for four days (Period 1), recording which individuals were present (27 ± 0.5 surveys per nest, mean $\pm$ standard error). Foraging effort was estimated as the proportion of surveys in Period 1 in which an individual was away from the nest.

Following [15], we identified the dominant (Rank 1) as the individual that was most often present on the nest. Where fewer than three surveys separated the closest contenders, we continued censusing each following day until this criterion was achieved. The next morning, Period 1 ended when we removed the Rank 1 wasp before 0800.

Censusing and successive removals continued until the ranks of all individuals had been ascertained (13–33 days), at which point the nest and remaining occupants were collected and frozen (-20°C). Inheritance rank estimates have a maximum error of one rank, because if one wasp died before its rank was known, we continued to estimate inheritance rank for the rest of the nest. If two wasps died, or if an individual died before the original dominant was identified, we did not estimate ranks any further for that nest. We obtained inheritance rank estimates for 177 subordinate wasps on 73 nests, and work effort data for 219 subordinates on 79 nests. 70 wasps died during the study period.

Nest defense. To assess whether unrelated subordinates participate in nest defense, we presented each nest with a dead conspecific "usurper" (from a distant site, killed by freezing) and filmed the reaction of the inhabitants, on sunny afternoons during Period 1. Usurpers were held with clean forceps, approximately 1 cm from the nest, for two minutes. We carried out assays on 75 nests.

Videos were scored using standard categories of aggression for this species [17]. We recorded "lunges" (leaping across nest, physical contact), "chews" (light biting), "grapples" (physical grasping) and "mounts" (climbing onto a nestmate). Subordinates' behaviour was classed as "aggressive" if they performed one or more of these acts towards the "usurper".

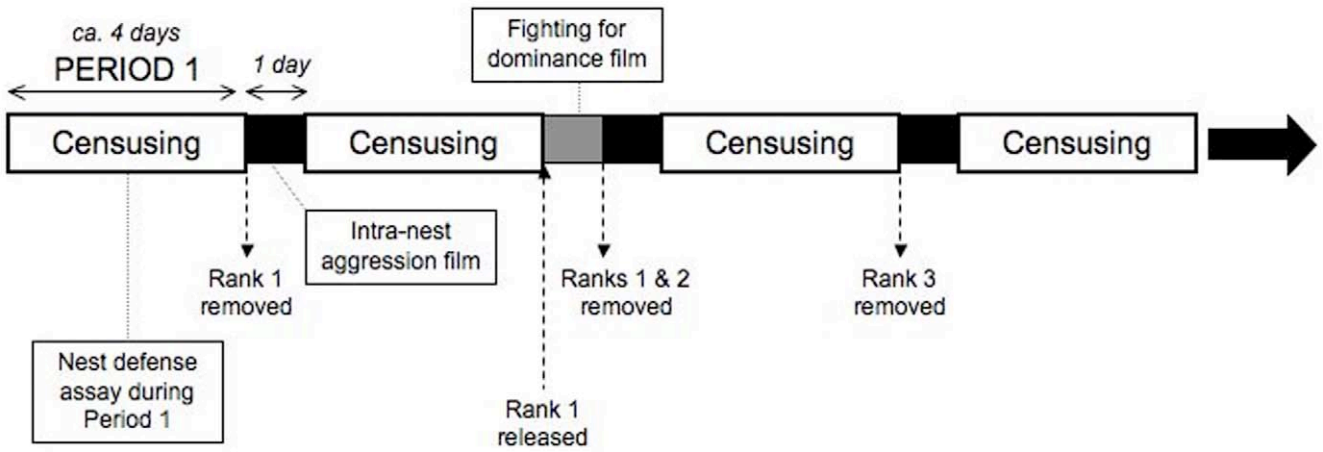

Figure 1. Sequence of experiments. Black/grey sections represent one day of no censusing. Observations continued until the rank of each wasp on the nest was known (pausing for bad weather).
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Intra-nest aggression. To assess whether unrelated subordinates initiate/receive more aggression from nestmates, we filmed nests for four hours on the afternoon following early-morning removal of the Rank 1 (Figure 1). Rank 1 removal is a key moment for subordinates, because it represents an opportunity for inheritance. Interactions were scored as described above. For each wasp, we calculated the mean number of aggressive acts initiated and received per hour present during the film (n = 139 wasps on 49 nests).

Fighting for dominance. Unrelated subordinates may have a greater incentive to fight the dominant for control of the nest, but escalated fighting is rarely observed in undisturbed colonies. Following [17], we induced fights by returning the Rank 1 to the nest four days after her removal at the end of Period 1, filming the subsequent interaction between her and the new dominant (the Rank 2). Before they were returned, Rank 1 foundresses were stored in the refrigerator at 5°C and fed with 50% (v/v) sugar solution every two days. They were released approximately 1 m from the nest, on sunny afternoons only.

We filmed the return of 50 Rank 1 wasps, classing fights as "escalated" if they lasted for more than 4 seconds, and/or if they included a "falling fight" whereby both wasps fall from the nest whilst grappling [17]. The following morning, both the Rank 2 and Rank 1 were permanently removed so that the ranks of remaining wasps could continue to be ascertained (Figure 1).

Relatedness estimation

Primer design. Microsatellite loci suitable for genotyping *P. dominulus* were identified by searching published literature and the EMBL sequence database. We used a selection of the primer sets previously isolated from *P. dominulus* and *P. bellicosus* [25,26]. Inspection of the 28 *P. dominulus* microsatellite sequences described by Henshaw [26] revealed that 23 consisted of multiple cloned inserts (as they contained multiple GATC restriction sites). Details of which loci failed to amplify and the primer sets for these are not provided by Henshaw, but any primer sets designed to amplify across two inserts would be expected to fail. We therefore designed three new primer sets to amplify sequences that contained just a single microsatellite-containing insert and these were found to amplify successfully. Details of all 8 primers, including the new *P. dominulus* primer sequences are provided in Text S1.

For each of the 8 loci, we tested for Linkage Disequilibrium (Gamete Disequilibrium Test), for deviations from Hardy-Weinberg Equilibrium (exact HW test) and for heterozygote deficiency (expected if null alleles are present, U test) in a sample of 64 non-relatives from our study, using the software Genepop 4.0 [27]. We found no significant deviations from chance expectations in all cases (p> Bonferroni-adjusted sequential p-values based on 0.05).

DNA extraction and amplification. To extract genomic DNA, wasp tarsi were bathed in 50 µl of buffer solution containing 10 mM Tris-Cl (pH 8.2), 1 mM EDTA, 25 mM NaCl, and 200 µg/ml Proteinase K (adapted from [25]). Samples were incubated at 57°C for 40 minutes, then at 95°C for 2 minutes, to inactivate the Proteinase K.

Multiplex polymerase chain reactions were performed on a Peltier Thermal Cycler, using 8 fluorescently labeled microsatellite primer pairs (amplified in a single multiplex set). Reactions of 4 µl were performed, containing approximately 80 ng of the template DNA, 0.75 µmol of three primer pairs (Pdom1jc, Pdom2jc and Pdom20), 0.375 µmol of the remaining five primer pairs (Pdom7, Pdom140, Pbe128TAG, Pdom127b and Pdom25), and 2 µl PEQlab hot start mix Y (1.25 u "Hot" Taq DNA polymerase per 25 µl, 0.4 nM dNTPs, 40 mM Tris-HCl, 32 mM $(NH_4)_2SO_4$, 0.02% Tween 20 and 4 mM $MgCl_2$). The temperature profile for the amplification was 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 57°C for 90 seconds and 72°C for 60 seconds; followed by a final extension step of 60°C for 30 minutes. A drop of mineral oil was added to prevent evaporation. Each plate included a positive and negative control to check for consistency of amplification.

PCR products were separated by size using a 48-well capillary Applied Biosystems 3730 Sequencer, compared with a size standard (Applied Biosystems GeneScan LIZ 500) and visualized using Applied Biosystems GeneMapper analysis software. We re-ran a subsample of 25 wasps to check for consistency of amplification and genotyping, and found that our genotyping error rate was low (only 1 incongruency between runs, in which a heterozygotic locus appeared homozygotic in one run).

Relationship assignment. We used the Full Sibship Reconstruction procedure in the program Kingroup (www.kingroup.org [28]) to establish each subordinate's relationship to the dominant. This procedure can divide groups of co-foundresses found on a nest into discrete sub-groups of full siblings- "sister groups"- based on the likelihood that all pairs of individuals within each sister group are sisters, and all pairs in different sister groups are related at the level of cousins or less. For example, consider a group of four co-foundresses that comprised two sisters, one cousin of these sisters and one unrelated wasp. The program would place the two sisters within the same sister group, and the cousin and unrelated wasps in two separate groups, producing three groups in total. Kingroup's allocation of pairs to "sister" or "cousin" categories is based on the likelihood that the genotypes of the two individuals would occur if they were full sisters, versus the likelihood that they would occur if the individuals were maternal cousins, given the population allele frequencies [29]. We provide a description of the iterative steps of the Full Sibship Reconstruction procedure that Kingroup uses in Text S2.

One we had divided cofoundresses from each nest into sister groups, we used the same procedure to identify cousin groups. In this case, the program follows exactly the same steps, but finds the pairwise likelihoods that individuals are cousins *vs.* unrelated. Thus, to summarize, for each nest, we knew which wasps were likely to be sisters of one another, which were likely to be cousins, and which were unrelated. Since we knew the dominant's identity, we could hence classify all subordinate wasps as sisters, cousins or non-relatives of the dominant wasp, and these three categories were used as predictors of behaviour in all subsequent analyses. However, since cousins and sisters most likely derive from the same natal nest, we also re-ran the same analyses where "sisters" and "cousins" were grouped as one category. Our findings did not change.

Statistical analyses

Behavioural data. Behavioural data were analyzed using the software R (2008 [30]). Our findings can be divided into two types of analyses: those where the data include more than one individual from each nest (individual foraging effort, responses to potential usurpers, and intra-nestmate aggression received/initiated), and those where each nest contributes only one value to the dataset (total nest foraging effort, total intra-nestmate aggression, occurrence of escalated fighting on return of Rank 1 wasp).

In the former case, data from individuals on the same nest cannot be considered independent, so we used mixed models where "nest" could be fitted as a random factor, to avoid pseudoreplication. For data where the error distribution of the variable being tested was expected to be binary (e.g. aggressive response *vs.* no response), we fitted generalized linear mixed models that assume a binomial error structure ("lmer"); otherwise, we fitted linear mixed effects models ("lme," suitable for a normal error distribution).

For those analyses that did not involve data from multiple wasps on the same nest, we used linear models. Again, where the response variable was binary, we used a model type suitable for a binomial error structure (generalized linear model, ''glm''); otherwise, we used linear models (''lm''). Proportional data (foraging effort) were arcsine transformed prior to analysis. A full list of the fixed effects included in each model, and a description of the model type for each behaviour tested, can be found in Text S3.

In each case, we began by fitting the full model, and proceeded by dropping the least significant terms sequentially until further removal led to a significant ($p<0.05$) decrease in the explanatory power of the model. This was assessed by comparing the models with and without the term in question, using Log-likelihood tests for linear mixed models (here the test statistic is a likelihood ratio L, which closely approximates a χ^2 distribution with v degrees of freedom, where v is the difference in the number of parameters between the two models), χ^2 values for models with a binomial error structure, and tabulated values of F-values for linear models with normal errors. To establish the final significance levels for each term, we added (non-significant) or removed (significant) terms to/from the minimal model. Non-significant terms ($p>0.05$) are not reported unless relevant to the main hypotheses.

We did not include inheritance rank as a predictor of behaviour in our analyses, because the rank of wasps on the same nest cannot be considered independent, since no position can be occupied by more than one wasp. However, since inheritance rank influences foraging effort and aggression [16,31,32], we first established that unrelated wasps did not occupy consistently different ranks to other subordinates (regression of relatedness to the Dominant against rank; Spearman's ρ). Inclusion of rank as a predictor of behaviour in the analyses did not alter the results. Data from foundresses of all ranks were included in the analyses.

Population relatedness. We carried out Maximum Likelihood analysis to ascertain the population composition, in terms of sister, cousin and unrelated pairs, that most closely matched the distribution of relatedness in our sample. Kingroup can produce distributions of pairwise relatedness values for simulated populations containing only individuals of a specified relatedness (e.g. sisters) based on user-defined population allele frequencies [28]. We created separate pools of haplodiploid sisters, cousins and non-relatives ($n>4000$ in each pool) based on our observed population allele frequencies, and then pseudo-randomly sampled from them to create relatedness distributions for populations of known composition. For example, to create the relatedness distribution of a population containing 75% sisters, 20% cousins and 5% non-relatives, we sampled pairwise relatedness values from the three pools in those proportions. We compared the 232 relatedness distributions created in this way (the composition of sisters, cousins, and non-relatives varying at 5% intervals between distributions) to our observed distribution using Kolmogorov-Smirnov tests. The population where the match was closest was identified by the highest p-value.

Results

Relatedness estimation

The distribution of within-nest, pair-wise relatedness across our entire DNA-sampled population (241 nests) showed a large peak around the full haplodiploid sister value of 0.75, and a smaller, broad peak centered at approximately 0.1 (Figure 2a). This

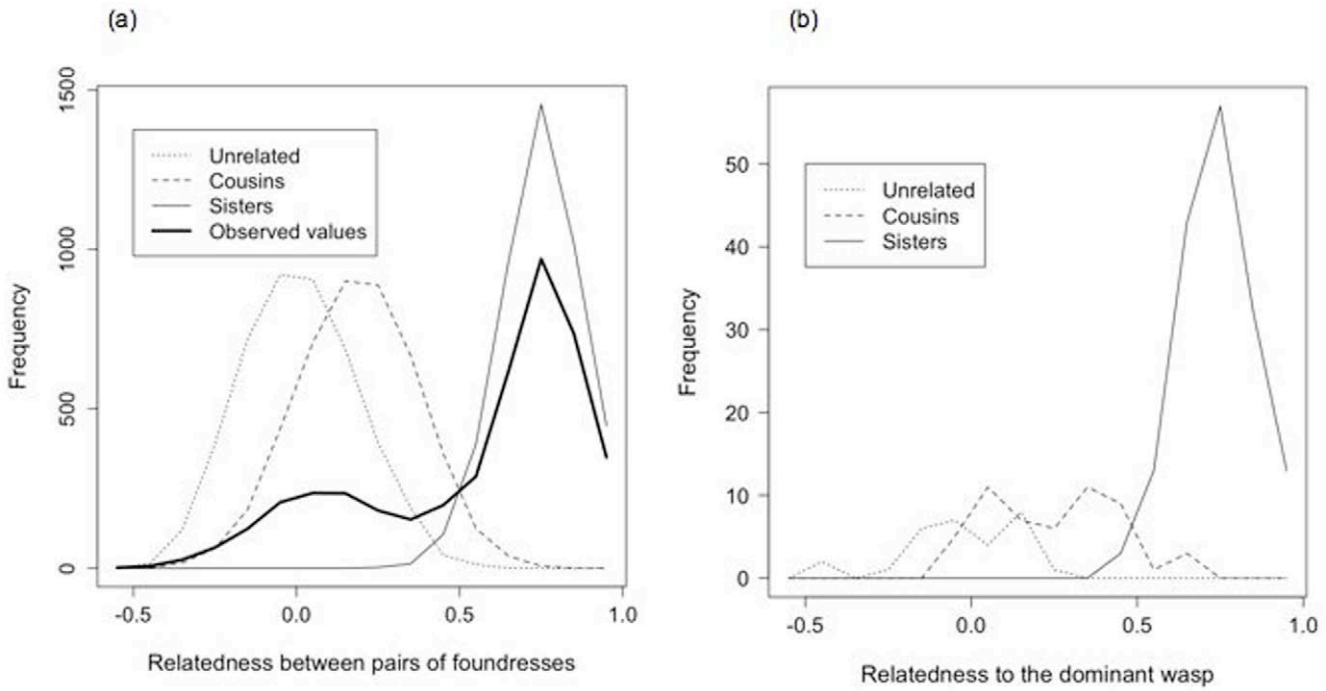

Figure 2. Relatedness in the study population and sample. a) Distribution of pairwise nest-mate relatedness across whole population, based on a sample of 4396 cofoundress pair (broad line). Further lines represent pairwise relatedness from simulated populations comprising 4396 pairs of sisters, cousins, and unrelated wasps b) Distribution of relatedness to the dominant wasp for subordinates classed as sisters of the dominant, cousins of the dominant, and non-relatives, on nests used for behavioural observations. Categories overlap because allocations are based on pairwise likelihoods, which depend on the population allele frequencies, and not absolute cut-off values. For example, an individual that is related to the dominant by less than 0.1 might be found to be more likely a cousin than unrelated, if the particular alleles that the two individuals do share are rare in the population.
doi:10.1371/journal.pone.0011997.g002

distribution is similar to that found previously in the Italian population studied by Queller et al. [4], although the proportion of non-sibling pairs is lower in our population. Maximum likelihood analysis reveals that our population most likely contains at least 15% unrelated pairs, with the remainder comprising 15% cousins and 70% full-sibling pairs. This population structure was more likely that any population containing 5% or fewer unrelated pairs by a factor of 1×10^3 and any population containing no unrelated pairs by a factor of more than 7×10^3. Thus, like the Italian population, our Spanish population contains a significant proportion of unrelated co-foundresses.

For the 72 nests used in behavioural observations, 12% of subordinates were classed as unrelated to the dominant, 66% as sisters of the dominant, and 22% as cousins (Figure 2b). 24.7% of nests contained at least one subordinate that was unrelated to the dominant wasp, and mean within-nest relatedness was 0.54 ± 0.015 (mean $\pm$ standard error). Nests containing unrelated foundresses did not differ significantly in number of co-foundresses from those containing only one sister group ($t_{76}=0.09$, $p=0.92$). Wing length (as a proxy for body size) did not differ significantly between sisters, cousins, and non-relatives of the dominant (means $\pm$ standard error: sisters: 11.7 mm ±0.06, cousins: 11.6 ± 0.09, unrelated: 11.72 ± 0.13, lme: $L_2=0.36$ $p=0.91$).

Behavioural data

Inheritance rank. We found no significant correlation between subordinates' inheritance ranks and relatedness to the dominant wasp on the nest (Figure 3, Spearman's $\rho=-0.11$, $p=0.13$). The dominant position was occupied by wasps with no relatives in the group no more often than would be expected by chance (χ^2 (Yates' correction) $=0.02$, d.f. $=1$, $p>0.01$).

Work effort. The total work effort on nests containing unrelated subordinates did not differ from nests that contained only sisters and cousins of the dominant (lm, $F_{1,75}=0.03$, $p>0.85$). Only the date (work levels dropped later in the season) and, as expected, group size (larger nests had higher total work effort) significantly influenced total work effort on a nest ($F_{1,76}=9.91$ and $F_{1,76}=99.32$ respectively, $p<0.01$ in both cases).

For individual wasps, a subordinate's relationship to the dominant wasp did not significantly influence work effort (Figure 4, lme: $L_1=0.003$ $p>0.95$). Again, wasps worked harder earlier in the season (lme: $L_1=6.87$, $p<0.1$), and when in smaller groups (lme: $L_1=4.02$, $p<0.05$). Including inheritance rank in the analysis (but see Methods) did not change these results.

We observed instances where aggression from a nestmate immediately preceded departure on 17 nests, but this was no more likely to occur on nests containing unrelated subordinates than other nests (χ^2 (Yates' correction) $=-1.75$, d.f. $=1$, $p>0.9$).

Nest defense. 80% of wasps that were present during the assay defended their nest. We found no significant differences between unrelated subordinates, cousins, and sisters of the dominant (lmer: $\chi^2=2.80$, d.f. $=1$, $p=0.08$). Of the three groups, cousins of the dominant, rather than unrelated wasps, responded the least aggressively (Figure 5). Smaller wasps were more likely to respond aggressively (lmer, $\chi^2=13.74$, d.f. $=1$, $p<0.01$).

Intra-nest aggression. When the dominant was removed, the subsequent level of aggression (mean aggressive acts initiated per wasp, per unit time) as wasps re-established the social hierarchy was no higher on those nests that contained unrelated subordinates than other nests (lm: $F_{1,48}=0.01$, $p=0.94$, Figure 6). Wasps with no sisters in the group (i.e. unrelated group members) neither received (lme: $L_1<0.00$, $p=0.99$) nor initiated (lme: $L_1=3.09$, $p=0.08$) significantly more aggressive acts than other wasps. Aggression that led recipients to temporarily leave the nest was rare (17 instances in over 1200 hours of video footage) and was no more likely to occur on nests containing unrelated subordinates than other nests (χ^2 (Yates' correction) $=-1.75$, d.f. $=1$, $p>0.9$).

Fighting for dominance. When fights between Rank 1 and Rank 2 wasps were experimentally induced, unrelated Rank 2 subordinates escalated conflict no more often than other wasps (glm: $\chi^2=2.08$, d.f. $=1$,$p=0.15$). When escalated fighting took place, the returning Rank 1 won the fight in all but a single case, on a nest where the Rank 1 and Rank 2 were sisters.

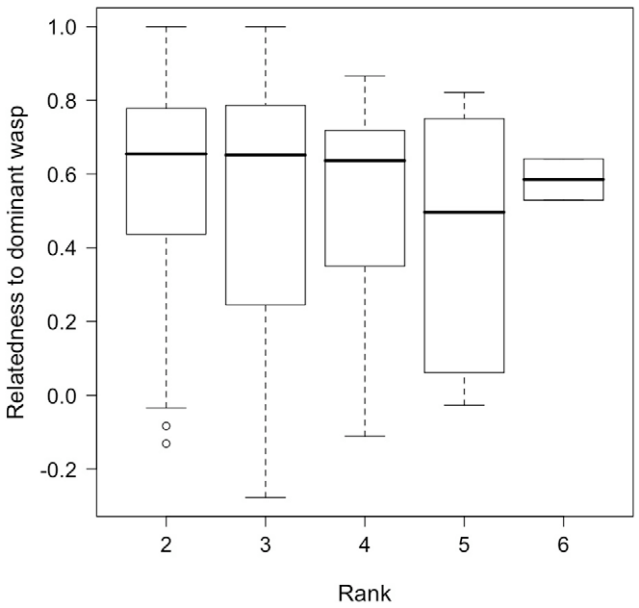

Figure 3. Inheritance rank in relation to a subordinate's relatedness to the dominant wasp. Medians, interquartile range and max/min values are indicated.
doi:10.1371/journal.pone.0011997.g003

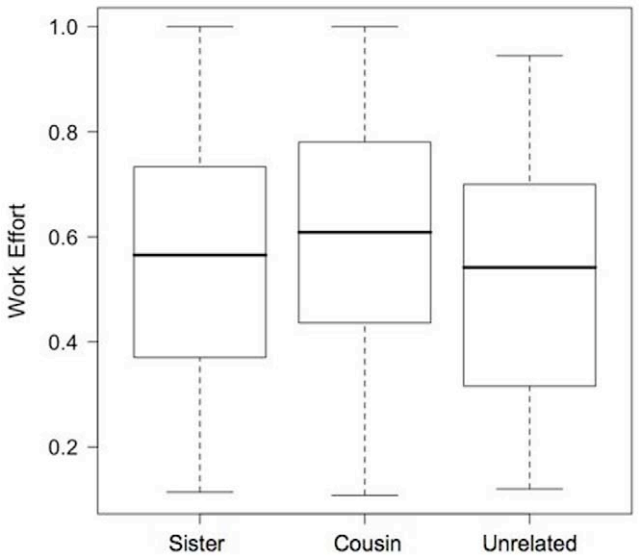

Figure 4. Foraging effort of sisters, cousins and non-relatives of the dominant wasp. Foraging effort is estimated based on proportion of time spent away from the nest.
doi:10.1371/journal.pone.0011997.g004

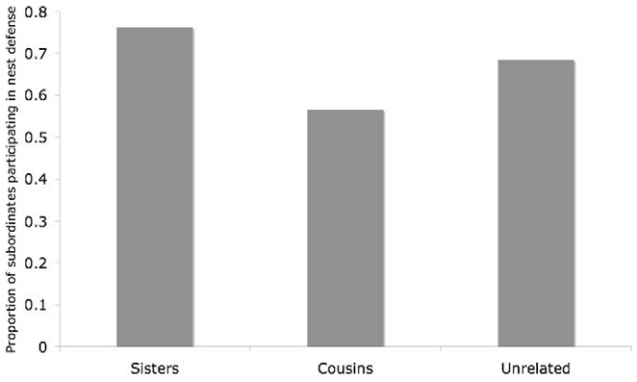

Figure 5. Aggressive responses to a conspecific usurper by sisters, cousins and non-relatives of the dominant.
doi:10.1371/journal.pone.0011997.g005

Discussion

Unrelated subordinates behaved like other members of *P. dominulus* social groups, and their presence did not affect group function across a wide range of contexts, in a natural habitat, with large sample sizes. Thus, we found no evidence that helping investment reflected a subordinate's relationship to the dominant wasp.

Our data provide no support for the hypothesis that we set out to test- that the helping behaviour of unrelated subordinates is tailored towards maximizing future fitness in *P. dominulus*. Given that reproduction through inheritance represents the only source of fitness for unrelated subordinates, what other hypotheses might explain why they raise unrelated brood? We discuss four alternatives.

First, selection to maximize direct fitness might have similar outcomes to selection to maximize indirect fitness. In other words, the same behaviours that boost the fitness of the current brood (and thus indirect fitness, for relatives of the dominant) may also boost future fitness. To re-visit an example discussed in the introduction, foraging to feed the dominant's brood might boost future fitness, by increasing group size and thus the helper's survival prospects. However, in our study, we specifically included behavioural contexts for which there is evidence that the fitness interests of unrelated and related subordinates are not aligned. We know from previous work that higher ranked wasps, that have greater expected future fitness, forage less [9,15], behave more aggressively towards nestmates [17], and challenge the dominant for control of the nest [18]. This provides a strong basis to suggest that the same trends should be apparent in the behaviour of unrelated subordinates, which are also under selection to maximize direct fitness.

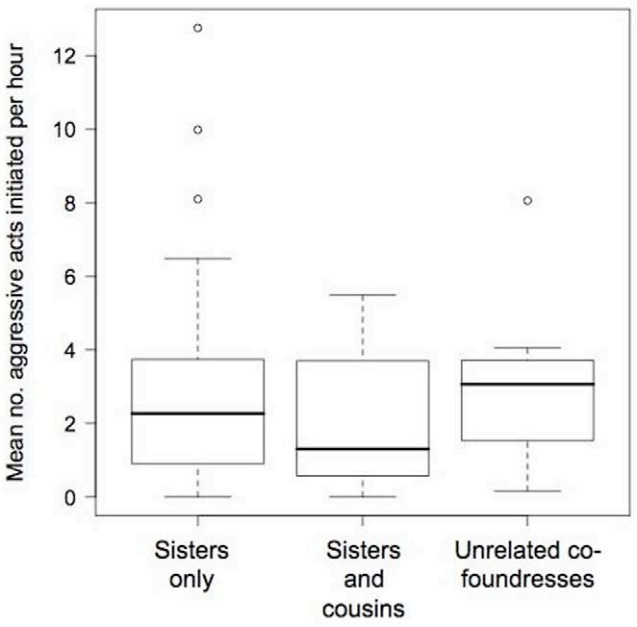

Figure 6. Aggression levels within founding groups. Mean aggression rates on nests where co-foundresses were all sisters, cousins and sisters, or contained at least one wasp that was not related to the rest of the group are shown.
doi:10.1371/journal.pone.0011997.g006

A related possibility is that relatives of the dominant may stand to gain little indirect fitness through raising spring brood, which contains a high proportion of non-reproductive workers [1]. Thus, both related and unrelated subordinates might be under selection to maximize future fitness, and we might see little difference in their behaviour. However, a substantial proportion of spring brood do indeed reproduce, because on some nests all foundresses die (approximately 23%, Leadbeater and Field unpublished data) and a worker can thus attain the dominant position [1]. In addition, many spring brood are male offspring, and thus reproductive [1]. Relatives of the dominant hence stand to gain indirect fitness through helping even on spring nests, albeit relatively less than on summer nests.

A third alternative is that unrelated helpers are not free to choose their own level of help, but must "pay" for group membership [33]. The dominant may be selected to evict unrelated helpers who might otherwise inherit in place of a relative, unless their elevated work effort justifies their presence. Can dominants evict subordinates, or otherwise enforce helping, in *P. dominulus*? Aggression that immediately preceded a subordinate leaving the nest to forage was rare in our study, and was equally directed towards relatives and non-relatives. However, perhaps actual evictions are not observed because the threat of eviction effectively motivates helping behaviour. Put differently, perhaps unrelated subordinates *would* be evicted if they did not work hard enough, but because this threat is effective, they do work hard and we do not see evictions [34]. Nonetheless, if this were the case, we should expect unrelated subordinates to work harder than relatives of the dominant, because the cost of their presence (a place in the inheritance queue that could otherwise have been occupied by a related subordinate) is higher. Thus, while our findings do not support the hypothesis that subordinates freely choose to maximize future fitness by working *less* hard, nor are they consistent with the hypothesis that unrelated subordinates pay-to-stay.

A final alternative is that helpers may make kin recognition errors. If unrelated co-foundresses derive from the same natal nest as their co-foundresses, kin recognition may be challenging. As we highlight in the introduction, although intranidal kin discrimination is rare in social insects, internidal discrimination is common [10], and at least some unrelated subordinates must derive from different natal nests to their co-foundresses [4]. Why should individuals not be capable of recognizing these outsiders as non-relatives? A possibility is that the hydrocarbon profiles of wasps overwintering together may become indistinguishable by the spring [35], since winter refuges are sometimes shared with individuals from other nests [36]. Relatedness in spring nests is not lower than within hibernaculae [5], suggesting that unrelated foundresses could plausibly be hibernaculum-mates of their co-foundresses, but this raises the question of why other *Polistes* do not

share this problem [37]. Nonetheless, kin recognition errors between hibernaculum-mates provide a plausible explanation for our findings, and further investigation of the source of unrelated foundresses is already underway.

Supporting Information

Text S1 Primer sequences. Details of primers used in this study, including three new primer sets.
Found at: doi:10.1371/journal.pone.0011997.s001 (0.05 MB DOC)

Text S2 Full Sibship Reconstruction procedure. Description of the iterative procedure followed by Kingroup during Full Sibship Reconstruction.
Found at: doi:10.1371/journal.pone.0011997.s002 (0.29 MB DOC)

Text S3 Statistical models. Descriptions of fixed effects and model types for each statistical model.
Found at: doi:10.1371/journal.pone.0011997.s003 (0.10 MB DOC)

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Author Contributions

Conceived and designed the experiments: EL JF. Performed the experiments: EL JMC JPG JvH. Analyzed the data: EL JMC. Contributed reagents/materials/analysis tools: JF. Wrote the paper: EL.

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Nest Inheritance Is the Missing Source of Direct Fitness in a Primitively Eusocial Insect

Ellouise Leadbeater,[1]*† Jonathan M. Carruthers,[1,2] Jonathan P. Green,[1] Neil S. Rosser,[3] Jeremy Field[1]

Animals that cooperate with nonrelatives represent a challenge to inclusive fitness theory, unless cooperative behavior is shown to provide direct fitness benefits. Inheritance of breeding resources could provide such benefits, but this route to cooperation has been little investigated in the social insects. We show that nest inheritance can explain the presence of unrelated helpers in a classic social insect model, the primitively eusocial wasp *Polistes dominulus*. We found that subordinate helpers produced more direct offspring than lone breeders, some while still subordinate but most after inheriting the dominant position. Thus, while indirect fitness obtained through helping relatives has been the dominant paradigm for understanding eusociality in insects, direct fitness is vital to explain cooperation in *P. dominulus*.

Primitively eusocial species provide a key testing ground for theories of the evolution of sociality, because helpers retain the ancestral ability to breed independently. In insects, such theories focus principally on indirect fitness acquired through aiding genetic relatives, because "sterile" workers in highly eusocial species have limited ability to reproduce (*1*, *2*). However, in the best-studied primitively eusocial system, *Polistes* paper wasps, indirect fitness has failed to fully explain group living (*3*, *4*). *Polistes dominulus* foundresses build new nests in spring, either alone or in small cofoundress groups, and survive for only a single, 5-month-long breeding season. On cofounded nests, subordinates forage to feed the larvae while one dominant individual lays almost all of the eggs (*5*). Inclusive fitness theory thus predicts high relatedness between cofoundresses (*6*), but surprisingly, 15 to 35% of *P. dominulus* subordinates in at least three populations are completely unrelated to the dominant wasp (*3*, *7*, *8*). These unrelated subordinates are the only social insects thought to eschew independent nesting to help raise the offspring of a nonrelative without obtaining inclusive fitness benefits in return (*7*).

In our study population, 15% of foundresses are unrelated to their cofoundresses, 15% are cousins, and the remainder are full sisters (*9*). The boost to group productivity provided by one subordinate is small enough that even full sisters of the dominant, who share 75% of her genes, would apparently do better to nest alone (*4*, *10*). However, these calculations have not taken into account the direct fitness to be gained from nest inheritance (*7*, *11*). Cofoundress associations are small in *Polistes*, and dominant turnover may be common (*4*, *7*, *12*, *13*), so that subordinates have a chance of inheriting the nest and its work force if the dominant dies or weakens before the breeding season ends (*7*). Indeed, variation in the chance of inheritance seems to drive individual variation in helping effort and aggression in *Polistes* and other primitively eusocial wasps (*12*, *14*, *15*). Studies that have focused on the early breeding season may thus have underestimated subordinate reproductive success [(*16*), but see (*17*)], but the value of inherited resources has rarely been quantified in social insects. Indeed, the relative contribution of direct fitness benefits to social evolution attracts heated debate, even in cooperatively breeding vertebrates where helping by nonrelatives is more common (*18*, *19*).

To evaluate whether subordinates outreproduce lone foundresses even in the absence of indirect fitness benefits, we measured the reproductive success of 1113 foundresses on 228 natural *P. dominulus* nests over the whole nesting season [mean cofoundress relatedness on collected nests = 0.53 ± 0.52 (SEM)]. In early spring 2008, we searched for foundresses that were initiating nests after emergence from hibernation at our study site in southern Spain. Each foundress was marked for identification, and a tarsal sample was taken (*17*) so that any pupae she produced could later be identified by genotyping. Cofoundress group composition fluctuates during the very early spring and stabilizes in late March, at which point we recorded the size of each group and identified the dominant wasp by behavioral censuses (*12*). To confirm the dominant's identity, we also genotyped the first offspring produced (*20*).

Because adult offspring are produced continuously from May until July, it is impossible to estimate reproductive output by sampling at a single time point. Instead, we estimated the number of offspring that subordinates produce in each quarter of the breeding season. First, we collected a random subset of nests at the time when the eggs laid immediately after group stabilization were about to reach adulthood (the early spring collection). We then carried out three more collections, each timed so that the oldest pupae on collected nests had pupated immediately after the previous collection date (late spring, early summer, and midsummer phase collections). We genotyped all pupae on collected nests at nine microsatellite loci, to estimate the number of pupae that subordinates produce in each quarter of the season, for comparison with single foundresses.

Pupae were classified as subordinate offspring if their individual (male) or sibling group (female) genotype was inconsistent with maternity by the dominant wasp but matched that of one or more subordinates. Over half (55.7%) of nests failed through predation or loss of foundresses

[1]School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK. [2]Natural Environment Research Council (NERC) Biomolecular Analysis Facility, University of Sheffield, Sheffield S10 2TN, UK. [3]Department of Genetics, Evolution, and Environment, University College London, London NW1 2HE, UK.

*Present address: Institute of Zoology, Zoological Society of London, Regent's Park, London NW4 4RY, UK.
†To whom correspondence should be addressed. E-mail: ellouise.leadbeater@ioz.ac.uk

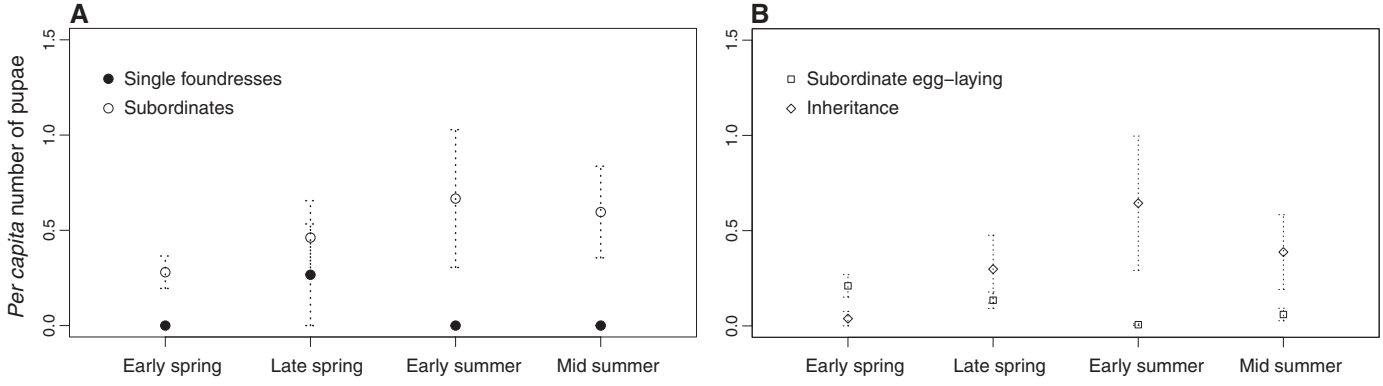

Fig. 1. Mean number of offspring produced by individual subordinates on each nest (per capita) after group stabilization (**A**) compared with lone foundresses (**B**) attained through egg-laying while still subordinate, and through inheriting the dominant position. Error bars mean ± SEM.

before their assigned quarter of the season, and in these cases each subordinate was recorded as having produced no pupae in that quarter. For each nest, we divided the total number of subordinate offspring by the number of subordinate foundresses to obtain subordinate offspring per capita. Remarkably, subordinates produced more direct offspring per capita than lone foundresses [$W = 4980$, $P < 0.001$ (*20*)], because they outreproduced lone foundresses in the latter part of the breeding season (Fig. 1A).

The disparity between lone foundresses and subordinates is greatest in the summer, when offspring are thought to be more likely to become next year's reproductives (*5*). We painted a date-specific mark on all 3072 female offspring that reached adulthood on a separate set of 145 nests, every 6 days from worker emergence until the season ended. In the spring of the following year, the vast majority (90%) of painted foundresses observed initiating spring nests originated from marking dates within the early and midsummer collections from the previous year (Fig. 2). The lone foundresses in our sample failed to produce a single pupa within this period.

Some subordinate offspring (32%) represented eggs laid while the dominant wasp was still alive, but the majority (68%) were produced after the subordinate had inherited the dominant position (Fig. 1B). Inheritance was not observed before the emergence of the nest's first offspring, but occurred most commonly immediately after this period (Table 1), suggesting that subordinates may challenge the dominant when the opportunity to lay offspring that are potential reproductives approaches. Based on the frequency of inheritance in each quarter, we estimate that on 87% of nests, the dominant will retain her position throughout the entire season, so the probability of inheritance for individual subordinates is low. Yet, the high payoff of inheritance, should it occur, means that an average subordinate gains more direct reproduction than an average lone foundress.

When inheritance occurs, the payoff to the inheritee is greater in larger cofoundress groups, because such nests are less prone to failure through predation or foundress death, especially early in the season (group size/collection date interaction: $\chi^2 = 7.65$, df = 1, $P < 0.01$) (fig. S1A). Furthermore, when they survive, nests founded by larger groups produce more pupae than their smaller surviving counterparts ($F_{1,96} = 13.0$, $P < 0.001$) (fig. S1B). However, in these larger cofoundress groups, each individual subordinate stands a lower chance of inheriting, because she faces greater competition from nestmates. Accordingly, subordinates produced the most offspring through inheritance per capita in medium-sized cofoundress groups (Fig. 3). However, we found no evidence that unrelated subordinates preferentially joined medium-sized groups (group size/relatedness correlation, Spearman's Rho: $P = 0.86$).

As well as sometimes inheriting the nest, foundresses could lay eggs while still subordinate. Subordinate egg-laying was more common in larger cofoundress groups ($F_{1,178} = 10.62$, $P < 0.01$) (Fig. 3). This might reflect enhanced difficulties in policing subordinate reproduction, or dominants in larger groups might allow more subordinate egg-laying to reduce the incentive for subordinates to fight for control of highly productive nests ("peace incentives") (*6*, *14*, *16*). Although subordinate egg-laying represented a smaller proportion (32%) of subordinate direct fitness than reproduction through inheritance (68%), even subordinates that do not inherit the dominant position could equal or exceed the reproductive output of lone foundresses (per capita reproduction through subordinate egg-laying versus lone foundress reproduction; $W = 3326$, $P < 0.01$) (Fig. 3).

Our analysis focused on average per capita subordinate reproduction, but it is possible that unrelated individuals (16.9% of subordinates in our sample) achieve less direct reproduction than others. To investigate this, we compared the per capita number of female pupae produced by subordinates that were relatives of the dominant (sisters or cousins), or were unrelated to her, across all nests that survived until collection. Overall, there were no significant differences ($P = 0.37$). We also found no relationship between mean cofoundress relatedness and the total productivity of the nest ($F_{1,84} = 1.73$, $P = 0.19$).

Our findings explain why only 4.04% of wasps chose to remain as lone foundresses at group stabilization (fig. S2): Individuals can

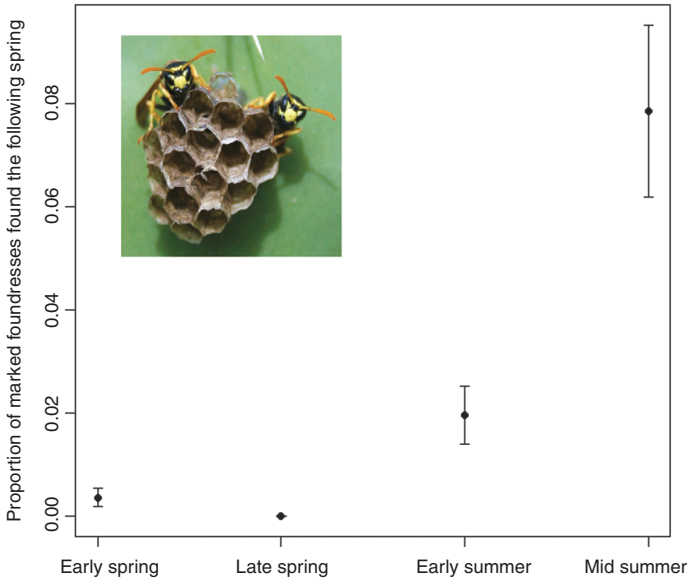

Fig. 2. Proportion of offspring produced during the early spring, late spring, early summer, and midsummer phases that were found on nests the following spring. Error bars, mean ± SEM. **(Inset)** *P. dominulus* cofoundresses on an early spring nest.

Table 1. Inheritance rates in each quarter of the season. Groups of nests were collected at the end of each quarter of the breeding season. If pupal genotypes indicated that a subordinate wasp was dominant at the start of that quarter, inheritance had occurred earlier in the season (column C). If the mother of the oldest pupal group was not the mother of the youngest, inheritance had occurred within that quarter (column D). Approximate dates when pupae were laid as eggs are based on egg/larval development times in (*22*) and pupal development times estimated by monitoring nests from our population, repeated three times over the season.

(A) Collection date (2008)	(B) Breeding season quarter	(C) Nests where inheritance occurred before pupal broods were produced (%)	(D) Nests where inheritance was observed within the pupal brood (%)
18 May	**Mid spring.** Pupae represent eggs laid before early May.	0	0
16 June	**Late spring.** Pupae represent eggs laid throughout May.	0	11.32
3 July	**Early summer.** Pupae represent eggs laid in early and mid-June.	9.76*	2.44
15 July	**Late summer**. Pupae represent eggs laid in late June and early July.	7.41	0

*One nest where the original dominant reinherited after the death of a usurper was not included in this statistic.

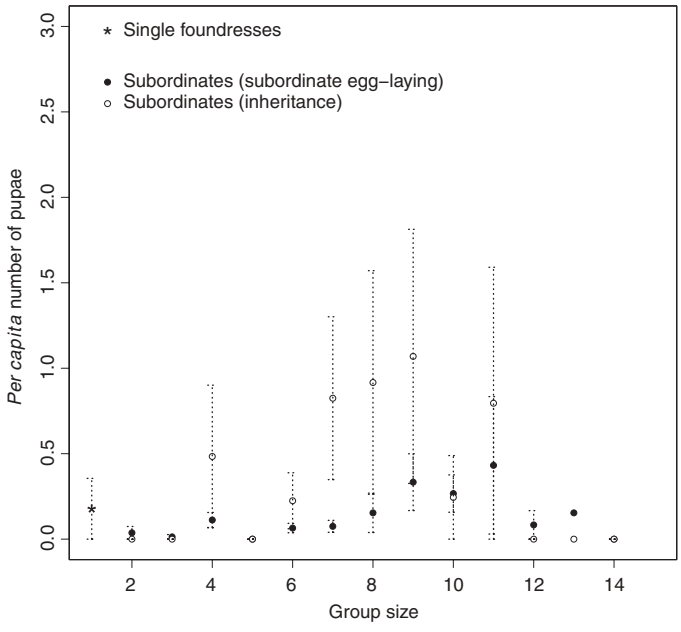

Fig. 3. Subordinate reproductive prospects, through subordinate egg-laying and inheritance, according to group size. Lone foundresses are illustrated for comparison. Error bars, mean ± SEM.

achieve more direct fitness through subordination even to a nonrelative than through nesting alone. However, we do not imply that direct fitness benefits are always the main driver of subordinate behavior, because our data also show that indirect benefits usually outweigh direct benefits for those subordinates [~56 to 70% (*7*, *9*)] that are relatives of the dominant wasp (fig. S3). Rather, direct fitness benefits make subordination worthwhile if wasps either do not have surviving relatives in the population or fail to recognize them. Within our sample, at least 12.8% of unrelated subordinates had sisters that were dominant on nearby nests, suggesting that kin recognition sometimes fails. Individuals should choose to nest with their sisters where possible, but the prospect of nest inheritance means that subordination can be adaptive even when this ideal cannot be achieved.

The importance of inheritance for *P. dominulus* subordinates, even within their short nesting season, means that like helpers in cooperatively breeding vertebrates, their behavior must reflect a trade-off between current (indirect) and future (direct) fitness (*12*, *14*). Inheritance has the potential to stabilize cooperation, because a dominant cannot easily accept help from subordinates, then later renege on the inheritance payoff after her own death (*21*). However, subordinate reproduction will also reduce relatedness between workers and egg-laying foundresses later in the season, helping to explain why a committed altruistic caste has not evolved in *Polistes* (*1*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/333/6044/874/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 and S2
References (*23*–*33*)

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Archaeorhizomycetes: Unearthing an Ancient Class of Ubiquitous Soil Fungi

Anna Rosling,[1,2*] Filipa Cox,[3] Karelyn Cruz-Martinez,[1] Katarina Ihrmark,[1] Gwen-Aëlle Grelet,[4] Björn D. Lindahl,[1] Audrius Menkis,[1] Timothy Y. James[5*]

Estimates suggest that only one-tenth of the true fungal diversity has been described. Among numerous fungal lineages known only from environmental DNA sequences, Soil Clone Group 1 is the most ubiquitous. These globally distributed fungi may dominate below-ground fungal communities, but their placement in the fungal tree of life has been uncertain. Here, we report cultures of this group and describe the class, Archaeorhizomycetes, phylogenetically placed within subphylum Taphrinomycotina in the Ascomycota. Archaeorhizomycetes comprises hundreds of cryptically reproducing filamentous species that do not form recognizable mycorrhizal structures and have saprotrophic potential, yet are omnipresent in roots and rhizosphere soil and show ecosystem and host root habitat specificity.

Direct sequencing of environmental DNA is a powerful tool to explore cryptic diversity of microorganisms and challenges our understanding of global biodiversity (*1*, *2*). Despite producing macroscopic reproductive structures and being among the largest of eukaryotes (*3*), many fungal species and even phyla have seldom been observed or cultivated (*4*–*6*). Among the lineages known only from environmental DNA sequences, the Soil Clone Group 1 (SCG1) (*5*) is the most common enigmatic lineage in soil (*7*, *8*). The mysterious nature of SCG1 stems from its detection by sequencing in more than 50 ecological studies of soil fungi (tables S1 and S2), but the organisms have never before been observed in the form of fruiting body, spore, culture, or distinctive

[1]Department of Forest Mycology and Pathology, Uppsala BioCentre, SLU, Box 7026, 750 07 Uppsala, Sweden. [2]Department of Biology, Indiana University, Bloomington, IN 47405, USA. [3]Imperial College London and Royal Botanic Gardens, Kew, London TW9 3DS, UK. [4]The University of Aberdeen and James Hutton Institute, Aberdeen AB24 3UU, UK. [5]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA.

*To whom correspondence should be addressed. E-mail: anna.rosling@slu.se (A.R.); tyjames@umich.edu (T.Y.J.)